PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07C 69/732, 233/22, A61K 31/216, 31/165

A1

(11) International Publication Number: WO 00/63152

(43) International Publication Date: 26 October 2000 (26.10.00)

(21) International Application Number: PCT/US00/04608

(22) International Filing Date: 22 February 2000 (22.02.00)

(30) Priority Data:
60/121,127 22 February 1999 (22.02.99) US

(71) Applicant (*for all designated States except US*): THE GOVERNMENT OF THE UNITED STATES OF AMERICA as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; 6011 Executive Boulevard, Suite 325, Rockville, MD 20852 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): BURKE, Terrence, R. [US/US]; 7400 Lakeview Dr., No. 410, Bethesda, MD 20817 (US). ZHAIWEI, Lin [CN/US]; 1113 Agnew Drive, Rockville, MD 20831 (US). ZHAO, He [CN/US]; 4 Stoneridge Lane, Brandford, CT 06405 (US). NEAMATI, Nouri [US/US]; 3721 Emily Street, Kensington, MD 20895 (US). POMMIER, Yves [US/US]; 8102 Maple Ridge Road, Bethesda, MD 20814 (US).

(74) Agent: GRESENS, John, J.; Merchant & Gould P.C., 3100 Norwest Center, 90 South Seventh Street, Minneapolis, MN 55402-4131 (US).

(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ACETYLATED AND RELATED ANALOGUES OF CHICORIC ACID AS HIV INTEGRASE INHIBITORS

(57) Abstract

Chicoric acid analogues and derivatives have activity against HIV-1 integrase. The structural features that are required for this activity are elucidated by assaying these analogues and derivatives against HIV-1 integrase. Furthermore, methods of synthesis of the enantiomers of chicoric acid itself, as well as its analogues and derivatives, are disclosed. Additionally, methods of use of chicoric acid analogues and derivatives to inhibit HIV-1 integrase are disclosed, as are compositions comprising chicoric acid analogues and derivatives.

X6

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

ACETYLATED AND RELATED ANALOGUES OF CHICORIC ACID AS HIV INTEGRASE INHBITORS

BACKGROUND

Infection with HIV leads to the disease AIDS. AIDS, or Acquired Immunodeficiency Syndrome, has become one of the most feared diseases of the last quarter of the twentieth century. Typically, AIDS strikes people during their most productive years, leading to illness and death. AIDS has become a serious health problem both in the developed countries and in the developing world. Although a number of treatments for AIDS have been developed, progress has been slow for a number of reasons. One of these reasons is the tendency of HIV to develop resistance or tolerance to drug treatments.

Treatment of AIDS using single agents often results in the development of tolerance. One way of potentially countering such tolerance is by employing combination therapies directed against different viral enzymes. To date, reverse transcriptase and protease inhibitors have been employed successfully in such a fashion; however availability of inhibitors directed against alternate targets is also desirable.

Integrase is a key enzyme in the viral life cycle which is required for replication. Lafemina et al., "Requirement of active human immunodeficiency virus type I integrase for productive infection of human T–lymphoid cells," J. Virol. 66, 7414–7419 (1992); Sakai et al., "Integration is essential for efficient gene expression of human immunodeficiency virus type 1," J. Virol. 7, 1169–1174 (1993); Taddeo et al., "Integrase mutants of human immunodeficiency virus type 1 with a specific defect in integration," J. Virol. 68, 8401–8405 (1994); Engelman et al., "Multiple effects of mutations in human immunodeficiency virus type 1 integrase on viral replication," J. Virol. 69, 2729–2736 (1995).

The integrase enzyme therefore offers an attractive target for anti–AIDS drug design, with such inhibitors being potentially complimentary to a multi–drug treatment regime. Erik De Clereq et al., "Toward improved anti–HIV chemotherapy: Therapeutic strategies for intervention with HIV infections," J. Med. Chem. 38, 2491–2517 (1995). For these reasons, significant effort has been

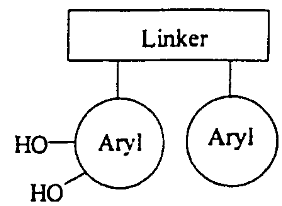
expended on the development of such agents. Pommier et al., "Review article: HIV−1 integrase as a target for antiviral drugs." Antivir. Chem. Chemother., 8, 463−483 (1997).

Integrase functions in a two step manner by initially removing a dinucleotide unit from the 3′−ends of the viral DNA (termed "3′−processing"), with the 3′ −processed strands then being transferred from the cytoplasm to the nucleus where they are introduced into the host DNA (termed "strand transfer" or "integration"). Radiolabeled oligonucleotide−based assays (Katz et al., "The avian retroviral IN protein is both necessary and sufficient for integrative recombination in vitro," Cell, 63, 87−95 (1990); Craigie et al., "A rapid in vitro assay for HIV DNA integration," Nucleic Acid Res., 19, 2729−2734 (1991)) which allow the *in vitro* determination of IC_{50} values for inhibition of both 3′ −processing and strand transfer have been utilized by several groups to examine the inhibitory efficacy of large numbers of agents. Neamati et al., "Design and discovery of HIV−1 integrase inhibitors," Drug Discov. Today, 2, 487−498 (1997). Using such assays, several classes of inhibitors have emerged, many of which contain multiple aromatic rings, with poly aryl−hydroxylation, frequently in the 1,2−catechol arrangement.

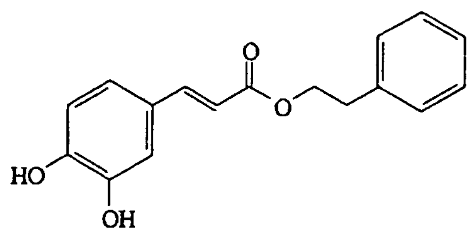
In many cases the aryl units of these inhibitors are separated by a central linker as typified by general structure A (see below). Among such inhibitors are flavones (Fesen et al., "Inhibition of HIV−1 integrase by flavones, caffeic acid phenethyl ester (CAPE) and related compounds," Biochem. Pharm., 48, 595−608 (1994); Kim et al., "A new flavonol glycoside gallate ester from Acer okamotoanum and its inhibitory activity against human immunodeficiency virus−1 (HIV−1) integrase," J. Nat. Prod., 61, 145−148 (1998)), arctigenins (Eckart et al., "(−)−Arctigenin as a lead structure for inhibitors of human immunodeficiency virus type−1 integrase," J. Med. Chem., 39, 86−95 (1996)), tyrphostins (Mazumder et al., "Effects of tyrphostins, protein kinase inhibitors, on human immunodeficiency virus type 1 integrase," Biochemistry, 34, 15111−15122 (1995)) and bis−catechols such as β−conidendrol. LaFemina et al., "Inhibition of human immunodeficiency virus integrase by bis−catechols," Antimicrob. Agents Chemother., 39, 320−324 (1995). Another group of related inhibitors is based on caffeic acid phenethyl ester (CAPE, structure B below) (Fesen et al., supra), and structural analogues such as structure C below. Burke et al., "Hydroxylated aromatic inhibitors of HIV−1 integrase," J. Med.

Chem., 38, 4171–4178 (1995); Zhao et al., "Arylamide inhibitors of HIV-1 integrase," J. Med. Chem., 40, 1186–1194 (1997). Even though catechols as a group exhibit favorable inhibitory profiles in cell-free integrase assays, often these compounds have failed to exhibit antiviral activity due to dose limiting toxicities in *in vitro* assays.

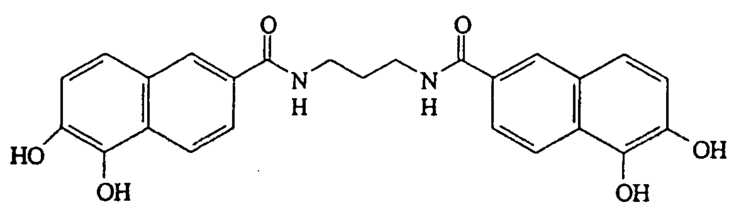
A:



B:



C:

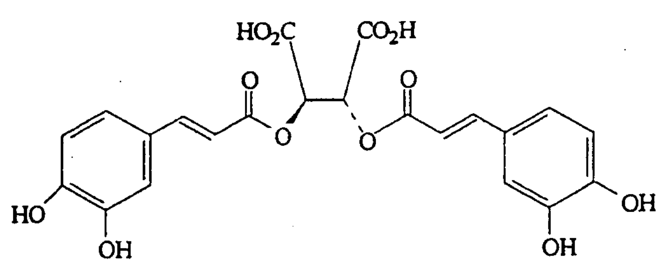


Recently, we have demonstrated that CAPE-like analogues can lead to crosslinking of intracellular proteins. Stanwell et al., "Cell protein cross-linking by erbstatin and related compounds," Biochem. Pharmacol., 52, 475–480 (1996). Toxicities may reflect oxidation of the catechols to reactive quinone species, accounting for the observed losses in cell viability. Thus, development of integrase inhibitors either lacking the catechol moiety or modifications which overcome its toxic properties has been the focus of considerable work. Neamati et al., supra.

It has been shown that compounds such as 4,5–di–O–caffeoylquinic acid which have multiple caffeoyl groups appended to a carboxylic acid–containing framework, can inhibit HIV replication in cell–based systems, presumably by interference with gp120–mediated binding of virus to the CD4 receptor. Mahmood et al, "Inhibition of HIV infection by caffeoylquinic acid derivatives," Antivir. Chem. Chemother., 4, 235–240 (1993). More recent work has reported that dicaffeoylquinic acids and the related compound, L–chicoric acid (dicaffeoyltartaric acid, structure D below), not only inhibit HIV–1 replication, but are also potent inhibitors of HIV–1 integrase. Robinson et al., "Inhibitors of HIV–1 replication that inhibit HIV integrase," Proc. Natl. Acad. Sci., 93, 6326–6331 (1996). Furthermore, it has been demonstrated that those agents are selective inhibitors of integrase when examined against a variety of other potential viral targets (McDougall et al., "Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type I integrase," Antimicrob. Agents Chemother., 42, 140–146 (1998)), and that they act on the catalytic core domain of the enzyme. Robinson et al., "Dicaffeoylquinic acid inhibitors of human innunodeficiency virus integrase: Inhibition of the core catalytic domain of human immunodeficiency virus integrase," Mol. Pharmacol., 50, 846–855 (1996).

More recently, resistance to antiviral effects of L–chicoric acid in HIV–1 infected cells has been achieved by a single mutation of integrase amino acid 140, supporting previous evidence that L–chicoric acid acts by inhibiting integrase, and that it does so by interacting near the enzyme catalytic triad. King et al., "Resistance to the anti–human immunodeficiency virus type 1 compound L–chicoric acid results from a single point mutation at amino acid 140 of integrase," J. Virol., 72, 8420–8424 (1998).

D:



The high potency of these bis–catechols in purified integrase is consistent with well established structure–activity relationship considerations. However, their reported efficacy in cell–based assays is remarkable in light of the failure of large numbers of structurally related compounds (for example, structures B and C, above). It was therefore of interest to examine structural features of L–chicoric acid (D) which are important for potency against the integrase, cellular toxicity and antiviral activity.

Therefore, there is a need for new derivatives of L–chicoric acid or analogous compounds that are potent inhibitors of HIV–1 integrase while maintaining antiviral activity and minimizing cellular toxicity. There is also a need for improved methods of treatment of HIV–1 using these compounds, alone or in combination therapy together with other anti–HIV–1 agents.

SUMMARY OF THE INVENTION

In order to meet these needs, we have developed new chicoric acid analogues and derivatives that inhibit HIV–1 integrase, and have elucidated the structure–activity relationships for these analogues and derivatives based on the activity exhibited by these new analogues and derivatives.

Another aspect of the invention is improved synthetic methods for enantiomers of chicoric acid itself, as well as its analogues and derivatives.

Still another aspect of the invention is the use of chicoric acid analogues and derivatives in *in vivo*, *ex vivo*, and *in vitro* methods for inhibiting the replication of HIV–1, either alone or together with other compounds that can inhibit the replication of HIV–1.

Yet another aspect of the invention is compositions comprising chicoric acid analogues and derivatives according to the invention.

The invention provides improved chicoric acid analogues and derivatives with activity against HIV−1 integrase. The chicoric acid analogues and derivatives of the present invention block both the 3′−processing and integration reactions of HIV−1 integrase.

The invention also provides improved methods for synthesizing both enantiomers of chicoric acid as well as chicoric acid analogues and derivatives.

Although the present invention has been described in considerable detail, with reference to certain preferred versions thereof, other versions and embodiments are possible.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

Figure 1 is a diagram depicting a pharmacophore, identified as "Pharmacophore A" as exemplary of a HIV−1 integrase inhibitor;

Figure 2 is a diagram depicting a scheme of solid−phase synthesis for a chicoric acid analogue;

Figure 3 shows a number of chicoric acid derivatives and analogues, their activity against integrase, their activity in the cytoprotection assay, and their cytotoxicity;

Figure 4 shows a number of additional chicoric acid analogues with a monocarboxylic structure, their activity against integrase, their activity in the cytoprotection assay, and their cytotoxicity;

Figure 5A is an autoradiograph of a denaturing 20% polyacrylamide gel showing the activity of various chicoric acid derivatives and analogues at a range of concentrations with the reactions run in Mn^{+2}; inhibitor concentrations in μM are indicated above each lane; electrophoresis is in a 20% denaturing acrylamide gel showing 19−mer 3′−processing products, the substrate 21−mer oligonucleotide, and higher molecular weight strand transfer (integration) products;

Figure 5B is an autoradiograph of a denaturing 20% polyacrylamide gel as in Figure 5A with the reactions run in Mg^{+2};

Figure 5C is a graph showing the quantification of the results of Figure 5A; inhibition was calculated after phosphorImager quantitation;

Figure 5D is a graph showing the quantification of the results of Figure 5B.

DESCRIPTION

I. CHICORIC ACID DERIVATIVES AND ANALOGUES

One aspect of the invention is chicoric acid derivatives and analogues. These chicoric acid derivatives and analogues are characterized by the following properties: (1) inhibitory activity against HIV−1 integrase; (2) antiviral activity against HIV−1 as measured in an assay that measures the effect of a compound in protecting a cell susceptible to HIV−1 infection from the cytopathic effect of HIV−1; and (3) minimal cellular toxicity.

The synthesis of these analogues and derivatives of chicoric acid is described below in Example 1; the testing of these analogues and derivatives is described below in Example 2.

The results provided below lead to the following conclusions about the structure−activity relationships of chicoric acid derivatives with respect to the inhibition of HIV−1 integrase:

(1) Removing the carboxylic acid moieties of the central linker and replacing them with methyl groups has only a minor effect on the inhibitory activity exhibited against HIV−1 integrase.

(2) Removing the carboxylic acid moieties of the central linker and joining them into a cyclohexane ring has only a minor effect on the inhibitory activity exhibited against HIV−1 integrase.

(3) Removing all substituents from the central linker, leaving the central linker as an ethylidene moiety, has only a minor effect on the inhibitory

activity exhibited against HIV−1 integrase. Because the removal of these substitutents eliminates chirality, the conclusion is that chirality is not required.

(4) If all substituents are removed from the central linker, increasing the length of the central linker from two carbons to three carbons, the activity against HIV−1 integrase decreases but is not abolished. This leads to the conclusion that, although two carbons is the preferred length of the central linker, the central linker can be three carbons or longer.

(5) If all substituents are removed from the central linker as in (3) and (4) above, replacing one of the caffeoyl ester linkages with an amide (−CONH) linkage, the activity against HIV−1 integrase decreases but is not abolished. This leads to the conclusion that, although it is preferred to have two caffeoyl ester linkages, at least one of the ester linkages can be replaced with an amide without abolishing activity.

(6) Acetylation of the four hydroxyl groups of the catechol moieties in the chicoric acids preserves activity against HIV−1 integrase.

(7) If the hydroxyls of the catechol moieties are substituted by acetylation, there must be at least one carboxyl function in the central linker. Removing these carboxyl functions substantially abolishes activity against HIV−1 integrase. In other words, either at least one carboxyl function in the central linker must be intact or the hydroxyls of the catechol moieties must be intact to preserve activity. Both of these structural features are not required to preserve activity, but one must be present.

(8) Removing one carboxyl function in the central linker preserves activity against HIV−1 integrase. Removing one carboxyl function in the central linker, leaving one carboxyl function remaining, still preserves activity even when one of the ester linkages between the central linker and the catechol moieties is replaced with an amide linkage. Similarly, removing one carboxyl function in the central linker and replacing both ester linkages between the central linker and the catechol moieties with amide linkages preserves the activity against the HIV−1 integrase. The activity of the molecule against HIV−1 integrase is substantially

preserved even though the hydroxyls of the catechol moieties are acetylated, although substitution of these hydroxyl moieties with methyl to produce tetramethyl ethers substantially abolishes activity. Moreover, the activity of the molecule against HIV−1 integrase is substantially preserved even though the carboxyl of the central linker is esterified with a methyl group. Thus, the charge of the carboxyl group is not required for activity.

In light of these conclusions, the following derivatives and analogues of chicoric acid are within the scope of the invention and are expected to have at least *in vitro* activity in inhibiting HIV−1 integrase:

(1) Analogues of the chicoric acids in which one or both of the carboxylic acid moieties of the central linker are replaced with lower alkyl groups. In this context, "lower alkyl" is defined to mean a branched or unbranched alkyl group of 1−6 carbons. Preferably, the lower alkyl group is from 1−3 carbons.

(2) Analogues of the chicoric acids in which one or both of the carboxylic acid moieties of the central linker are replaced with functional groups bearing a carbonyl function, such as acetyl or propionyl.

(3) Analogues of the chicoric acids in which the carboxylic acid moieties are removed and the central linker is included into a ring structure. The resulting ring can have the carbon skeleton of cyclohexane, cyclopentane, cyclohexene, cycloheptane, cycloheptene, or benzene.

(4) Analogues of the chicoric acids in which the central linker is three carbons in length.

(5) Analogues or derivatives of the chicoric acids in which one or both of the caffeoyl ester linkages is replaced with an amide linkage. Alternatively, the O of the ester or the NH of the amide can be replaced with a CH_2 moiety.

(6) Derivatives of the chicoric acids in which from one to four of the hydroxyls of the catechol moieties are esterified with an acyl group or converted into a carbamate by condensation with an appropriate isocyanate−containing moiety,

either alkyl or aryl, to form an alkyl carbamate or an aryl carbamate, respectively. The acyl group can be acetyl, propionyl, butyryl, or isobutyryl. If an alkyl carbamate is formed, the alkyl portion of the resulting carbamate can have from one to three carbons, referred to herein generically as "lower alkyl."

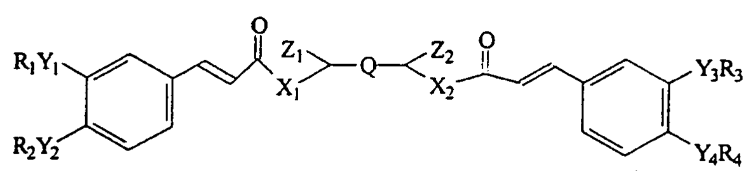
(7) Analogues of the chicoric acids in which one of the carboxylic acid groups of the central linker is replaced by hydrogen and the other carboxylic acid group is unmodified or is esterified with a lower alkyl group.

(8) Analogues of the chicoric acids in which the phenolic hydroxyls are replaced with amino moieties which are then condensed with an acetyl, propionyl, butyryl, or isobutyryl group, forming an amide.

(9) Analogues of the chicoric acids in which the hydroxyl groups of the catechol moieties are replaced with a carboxy-containing moiety such as carboxymethyl, carboxyethyl, or carboxypropyl, joined directly to the aromatic ring.

These relationships are summarized in formula (I) below, which schematizes chicoric acid analogues and derivatives studied, including the novel compounds according to the invention.

(I):



where:

Q is a valence bond or CH_2;

X_1 is O, NH, or CH_2;

X_2 is O, NH, or CH_2;

Y_1, Y_2, Y_3, and Y_4 are each a valence bond, O, or NH;

where Y_1, Y_2, Y_3, or Y_4 is a valence bond, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is a carboxy-containing moiety selected from the group consisting of carboxymethyl, carboxyethyl, carboxypropyl, carboxy small alkyl and carboxy aryl;

where Y_1, Y_2, Y_3, or Y_4 is O, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each H, acetyl, propionyl, butyryl, isobutyryl, or is a moiety forming a lower alkyl carbamate or an aryl carbamate;

where Y_1, Y_2, Y_3, or Y_4 is NH, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each acetyl, propionyl, butyryl, isobutyryl, small alkyl or aryl;

Z_1 and Z_2 are each H, lower alkyl, $-CHO$, $-CO_2H$, or $-CO_2W$, where W is lower alkyl or aryl, or, alternatively, where Q is a valence bond, Z_1 and Z_2, together with the adjacent carbon atoms and Q, form a ring structure, the carbon skeleton of the ring structure being selected from the group consisting of cyclohexane, cyclohexene, cyclopentane, cycloheptane, cycloheptene, and benzene;

with the proviso that where each of Y_1, Y_2, Y_3, or Y_4 is O and all of R_1, R_2, R_3, and R_4 are other than H, at least one of Z_1 or Z_2 is $-CO_2H$ or $-CO_2W$.

This formula includes both enantiomers where they exist. For L–chicoric acid and D–chicoric acid, previously known compounds, each of R_1, R_2, R_3, and R_4 is H, each of Y_1, Y_2, Y_3, and Y_4 is O, X_1 and X_2 are both O, Q is a valence bond, and Z_1 and Z_2 are both $-CO_2H$. Therefore, novel compounds according to the invention include compounds of formula (I) where:

Q is a valence bond or CH_2;

X_1 is O, NH, or CH_2;

X_2 is O, NH, or CH_2;

Y_1, Y_2, Y_3, and Y_4 are each a valence bond, O, or NH;

where Y_1, Y_2, Y_3, or Y_4 is a valence bond, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is a carboxy–containing moiety selected from the group consisting of carboxymethyl, carboxyethyl, carboxypropyl, carboxy small alkyl and carboxy aryl;

where Y_1, Y_2, Y_3, or Y_4 is O, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each H, acetyl, propionyl, butyryl, or isobutyryl, or is a moiety forming a lower alkyl carbamate or an aryl carbamate;

where Y_1, Y_2, Y_3, or Y_4 is NH, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each acetyl, propionyl, butyryl, isobutyryl, small alkyl or aryl;

Z_1 and Z_2 are each H, lower alkyl, $-CHO$, $-CO_2H$, or $-CO_2W$, where W is lower alkyl or aryl, or, alternatively, where Q is a valence bond, Z_1 and Z_2, together with the adjacent carbon atoms and Q, form a ring structure, the carbon skeleton of the ring

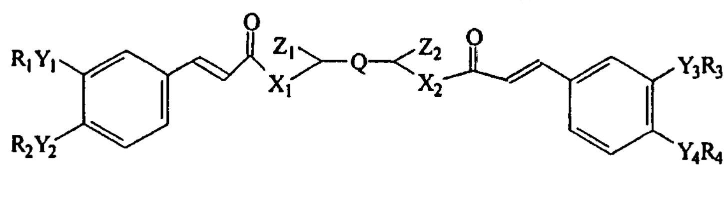
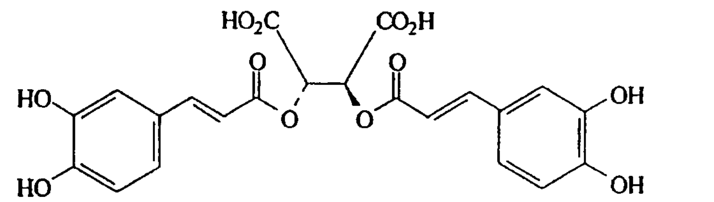
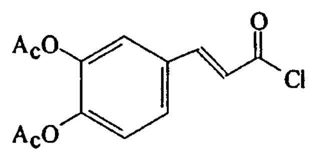
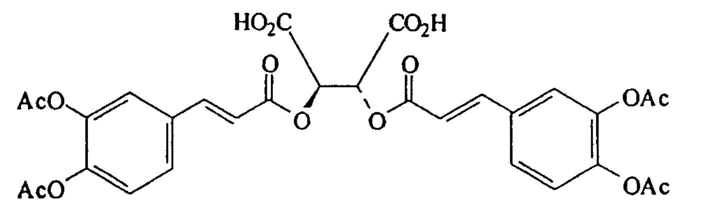
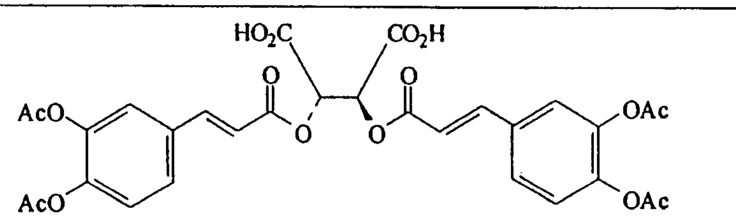
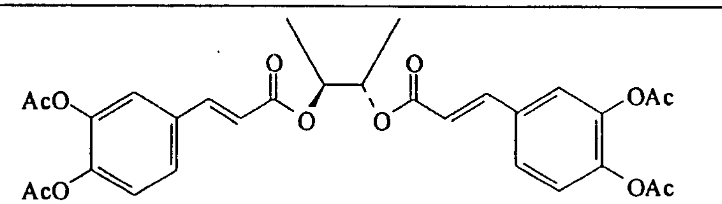
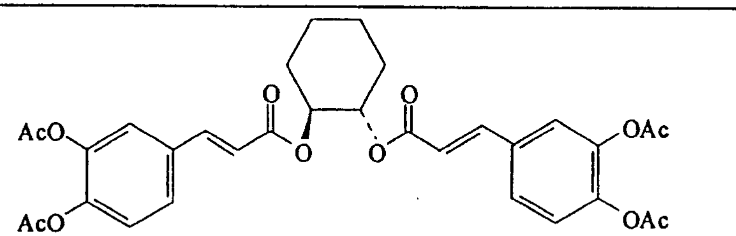
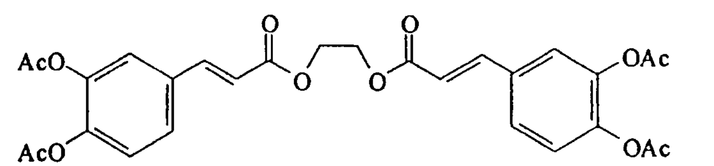
structure being selected from the group consisting of cyclohexane, cyclohexene, cyclopentane, cycloheptane, cycloheptene, and benzene; with the proviso that where each of Y_1, Y_2, Y_3, or Y_4 is O and all of R_1, R_2, R_3, and R_4 are other than H, at least one of Z_1 or Z_2 is $-CO_2H$ or $-CO_2W$; and with the proviso that where X_1 and X_2 are both O, Q is a valence bond, and Z_1 and Z_2 are both $-CO_2H$, either at least one of R_1, R_2, R_3, and R_4 is other than H or at least one of Y_1, Y_2, Y_3, and Y_4 is other than O.

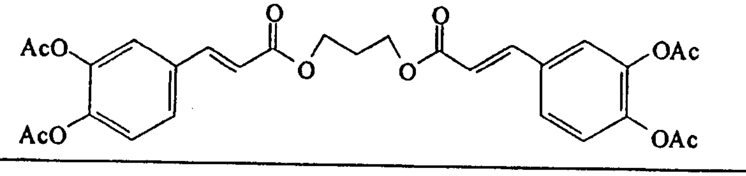
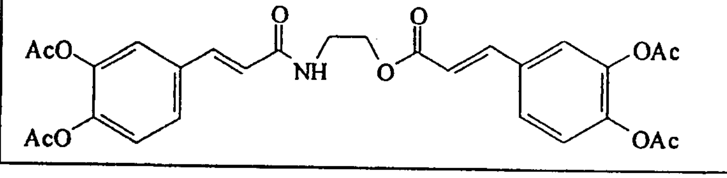
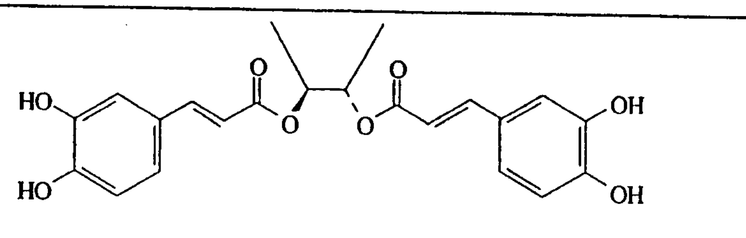
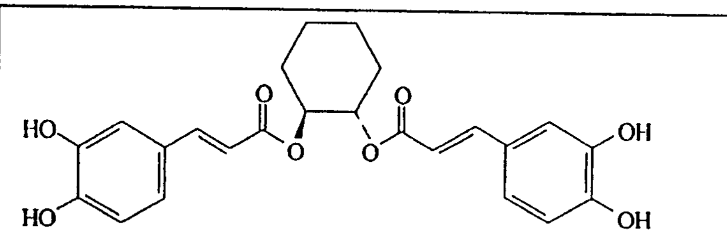
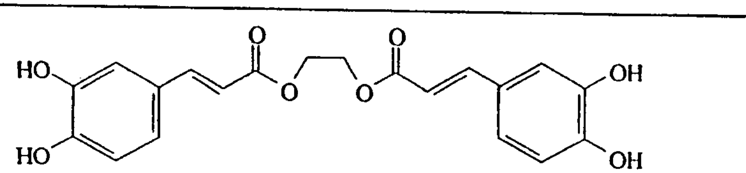
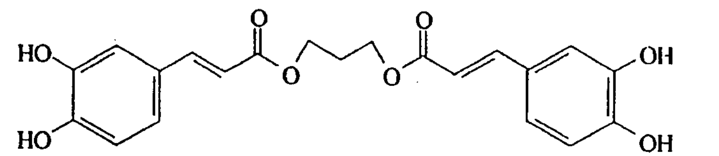
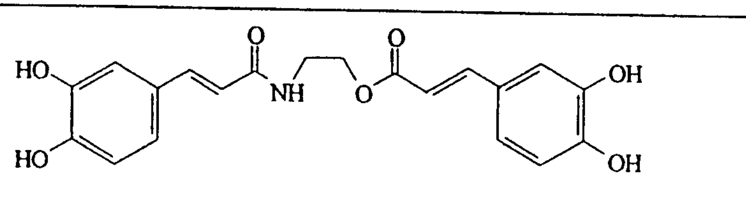
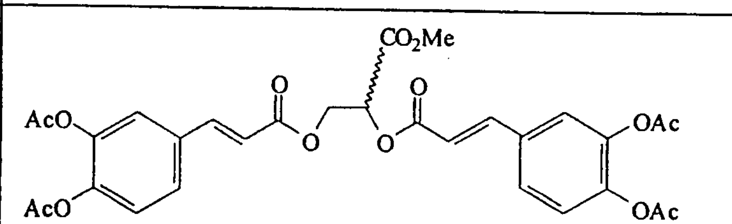
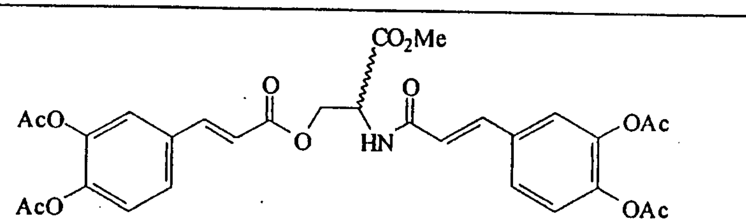
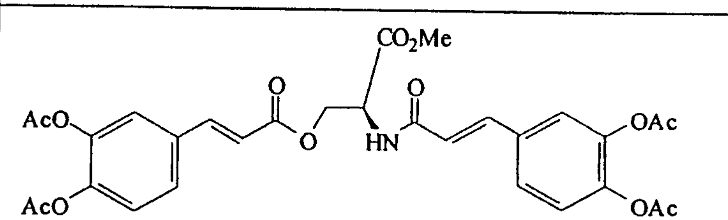
The novel chicoric acid analogues and derivatives (see Table 1) according to the present invention include the following:

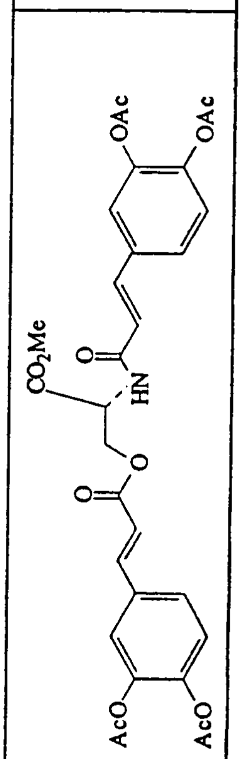
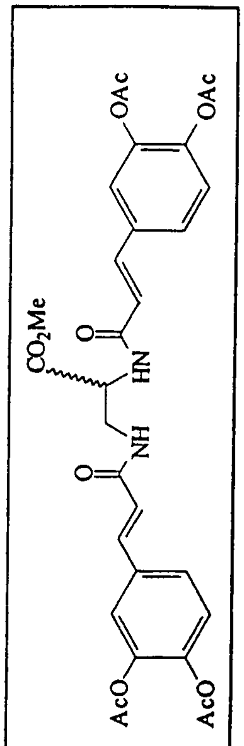
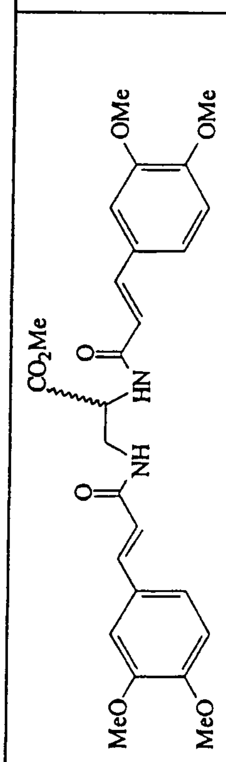
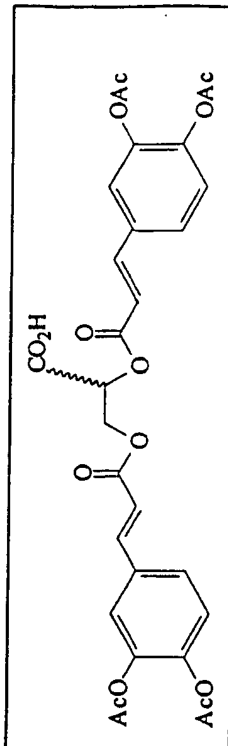
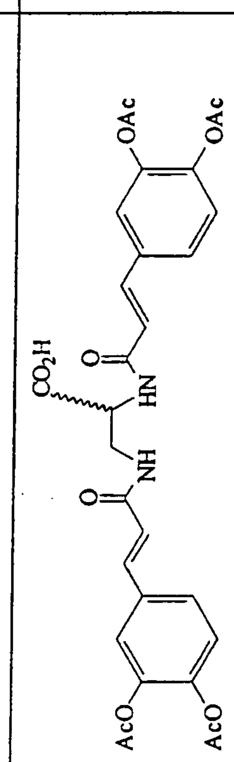
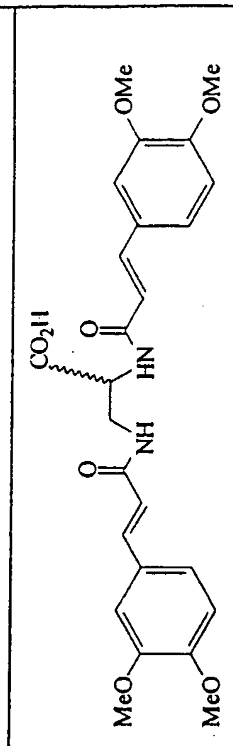
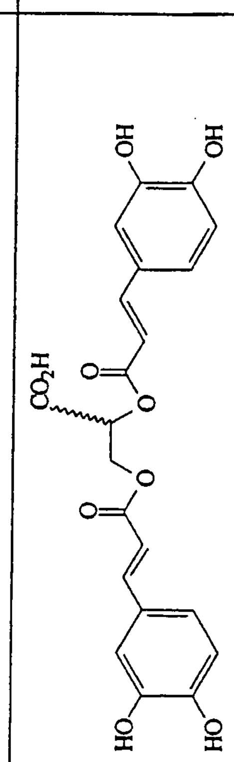
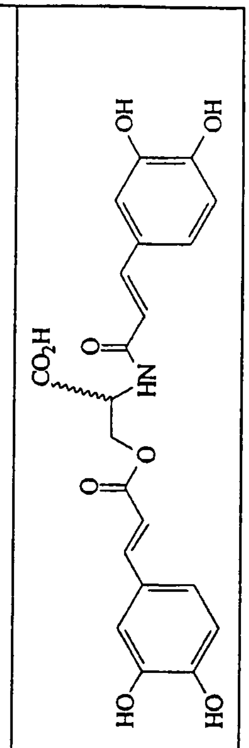
(1) L–chicoric acid tetraacetate (formula 6); (2) D–chicoric acid tetraacetate (formula 7); (3) (+)–(2S,3S)–O,O–bis–(3,4–dihydroxycinnamoyl)–2,3–butanediol (formula 13), in which the carboxylic oxygens of the central linker are removed, while both ester linkages are preserved; (4) (+)–(1S,2S)–O,O–bis–(3,4–dihydroxycinnamoyl)–1,2–cyclohexanediol (formula 14), in which the carboxylic oxygens of the central linker are removed and the central linker is incorporated into a cyclohexane ring, while both ester linkages are still preserved; (5) O,O–bis–(3,4–dihydroxycinnamoyl)–1,2–ethanediol (formula 15), in which the substituents are removed completely from the central linker, leaving a hydrocarbyl linker framed by ester linkages; (6) a monocarboxylic acid analogue prepared from glyceric acid, O,O–bis–(3,4–dihydroxycinnamoyl)–2,3–dihydroxypropanoic acid (formula 27); (7) a monocarboxylic acid analogue prepared from serine, in which one caffeoyl ester linkage is replaced by an amide linkage; N,O–bis–(3,4.dihydroxycinnamoyl)serine (formula 28); (8) a tetraacetate derivative of a monocarboxylate in which the caffeoyl groups were attached to the central linker by ester linkages, O,O–bis–(3,4–diacetoxycinnamoyl)–2,3–dihydroxypropanoic acid (formula 24); (9) a tetraacetate derivative of a monocarboxylate in which the caffeoyl groups were attached to the central linker by amide linkages, N,N–bis–(3,4–diacetoxycinnamoyl)–2,3–diaminopropanoic acid (formula 25); and (10) compounds that are derivatives of monocarboxylates in which the carboxyl group of the central linker is esterified with a methyl group, namely methyl O,O–bis–(3,4–diacetoxycinnamoyl)–2,3–dihydroxypropanoate (formula 18), methyl N,O–bis–(3,4–diacetoxycinnamoyl)serinate (formula 19), methyl N,O–bis–(3,4–diacetoxycinnamoyl)–L–serinate (formula 20), methyl N,O–bis–(3,4–

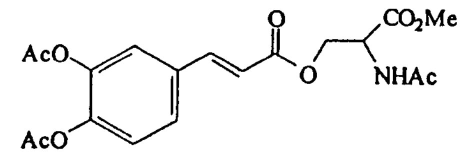
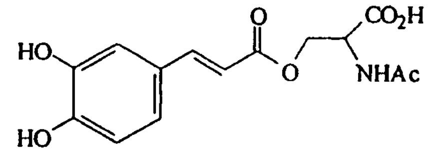
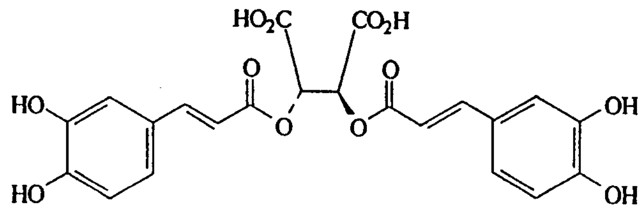
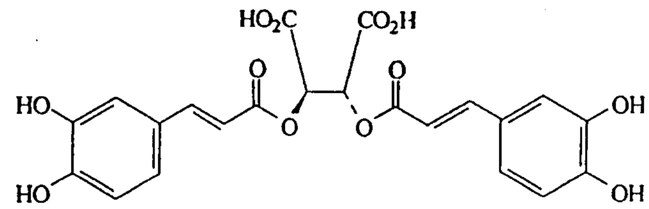
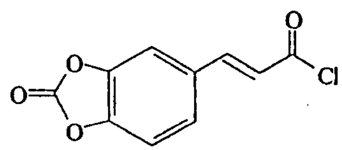
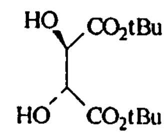
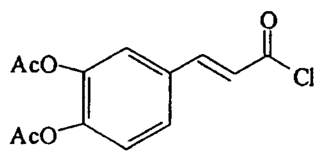
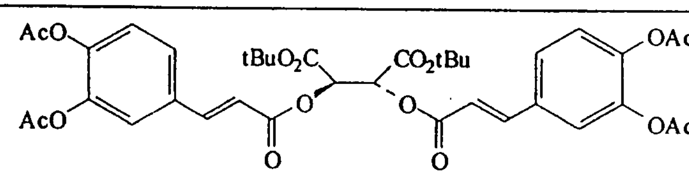
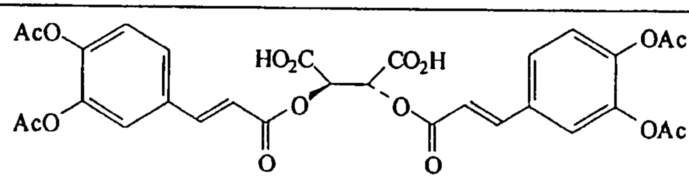
diacetoxycinnamoyl)–D–serinate (formula 21), and methyl N,N–bis–(3,4–diacetoxycinnamoyl)–2,3–diaminopropanoate (formula 22).

TABLE 1

	STRUCTURE		STRUCTURE
I		4	
5		6	
7		8	
9		10	

11		12	
13		14	
15		16	
17		18	
19		20	

21	
22	
23	
24	
25	
26	
27	
28	

29		30	
31		32	
33		34	
35		36	
37			

II. METHODS OF SYNTHESIS OF CHICORIC ACID DERIVATIVES AND ANALOGUES

Another aspect of the present invention is methods of synthesis of chicoric acid derivatives and analogues. These methods include both conventional synthetic methods and solid phase synthesis.

One method of synthesis of chicoric acid analogues and derivatives having two carboxyl groups in their central linkers, in general, comprises:

(1) reacting di–t–butyl tartrate with the acid chloride of a protected derivative of dihydroxycinnamic acid having its phenolic hydroxyl groups acylated;

(2) removing the di–t–butyl groups; and

(3) removing the acyl groups protecting the phenolic hydroxyls to produce the chicoric acid or chicoric acid analogue or derivative having two carboxyl groups in its central linker.

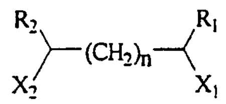
Specifically, a method of synthesis of both enantiomers of chicoric acid involves reaction of the enantiomers of di–t–butyl tartrate, (+)–di–t–butyl L–tartrate or (–)–di–t–butyl D–tartrate, with 3,4–caffeoyl acid chloride. This results in the formation of the di–t–butyl esters of chicoric acids, which can then be converted to the chicoric acids by trifluoroacetic acid–mediated hydrolysis of the esters. Other hydrolysis methods can be used to convert the esters to the chicoric acids.

Specifically, for the synthesis of (2R,3R)–(–)–chicoric acid, 1 mmol of (+)–di–t–butyl L–tartrate can be dissolved in 3 ml of anhydrous pyridine and treated with 2 equivalents of a solution of 3,4–caffeoyl acid chloride in toluene (5 ml to 1 mmol of the tartrate) at room temperature overnight. After removal of pyridine, the residue is dissolved in toluene and evaporated under reduced pressure. Two subsequent additions and evaporations of toluene eliminate residual pyridine. The resulting material is passed through silica gel (ethyl acetate:hexane, 1:1). then crystallized from ethyl acetate:hexane to provide the t–butyl diester as a white solid (97% yield). The t–butyl diester is then treated with trifluoroacetic acid (20 equivalents) in dichloromethane (v/v=1:3) at room temperature overnight, than either crystallized (acetone:hexane) to provide the free diacid with the four hydroxyls on the catechol moieties esterified as a white solid (96%), or taken directly to dryness and

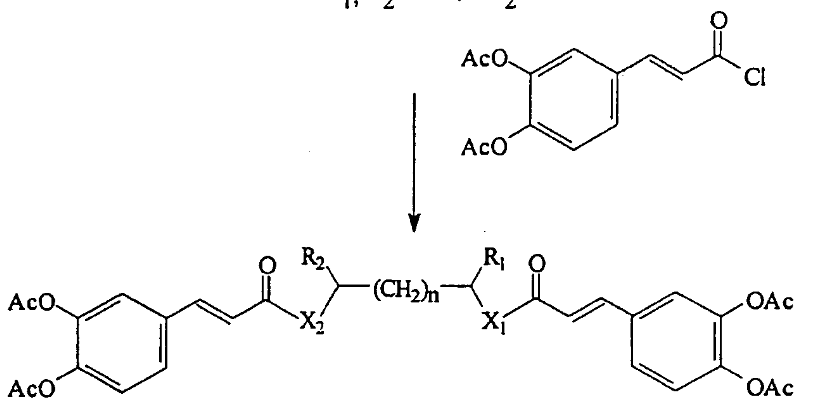
hydrolyzed with 3 N HCl (10 ml to 1 mmol of the diester) in acetone (v/v = 1:3) under reflux for 3 hours. After cooling to room temperature, the solution is diluted with 5 volumes of ethyl acetate, washed with brine, and dried over anhydrous sodium sulfate. Solvent is removed and residue crystallized from H_2O to provide (2R,3R)–(–)–chicoric acid in 90% yield. Enantiomeric natural (2S,3S)–(+)–chicoric acid can be obtained in an identical fashion starting from (–)–di–t–butyl D–tartrate. This method can be used for the synthesis of chicoric acid analogues and derivatives by selection of the appropriate esters and acid chlorides.

The method can be further generalized as shown in Example 1, below, and in Schemes 1 and 2. In one alternative of the generalized method, the acid chloride of a protected derivative of dihydroxycinnamic acid having its phenolic hydroxyl groups acylated is reacted with a diol or ethanolamine. This alternative forms the desired product directly. This alternative is shown in Scheme 1. In this alternative, the desired product has no carboxyl groups, and producing the desired product requires only removing the acyl groups protecting the phenolic hydroxyls to produce the chicoric acid analogue or derivative. The chicoric acid analogue or derivative thus produced has no carboxyl groups in its central linker.

Scheme 1:

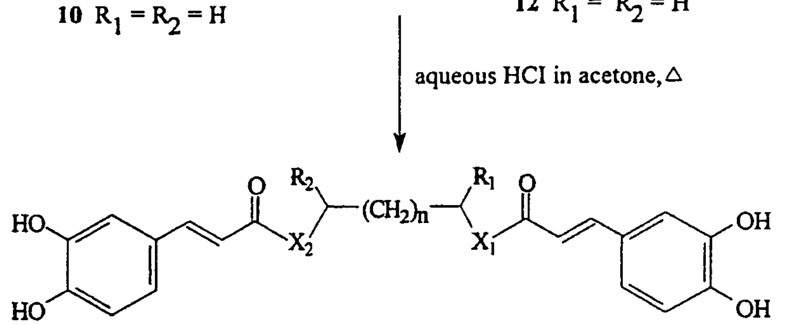


X_1, X_2 = OH, NH_2



for n = 0, $X_1 = X_2$ = O
8 $R_1 = R_2$ = Me
9 $R_1 = R_2$ = 1,2-cycloxhexane
10 $R_1 = R_2$ = H

for n = 1, $X_1 = X_2$ = O
11 $R_1 = R_2$ = H
for n = 0, X_1 = O, X_2 = NH_2
12 $R_1 = R_2$ = H

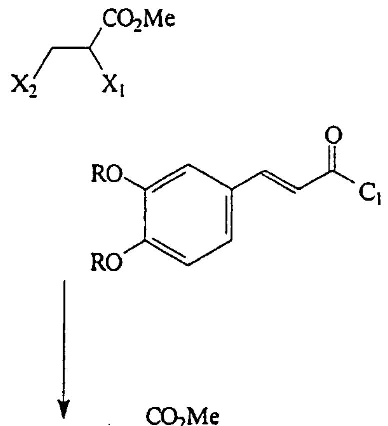


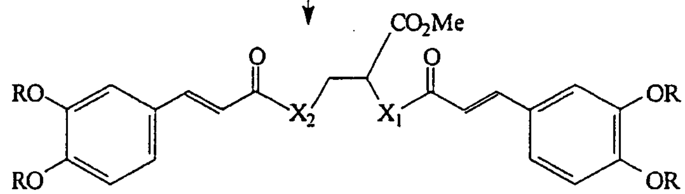
for n = 0, $X_1 = X_2$ = O
13 $R_1 = R_2$ = Me
14 $R_1 = R_2$ = 1,2-cycloxhexane
15 $R_1 = R_2$ = H

for n = 1, $X_1 = X_2$ = O
16 $R_1 = R_2$ = H
for n = 0, X_1 = O, X_2 = NH_2
17 $R_1 = R_2$ = H

In another alternative of the generalized method, the acid chloride of a protected derivative of dihydroxycinnamic acid having its phenolic hydroxyl groups acylated is reacted with a dihydroxy compound that is further substituted with an alkyl ester group, such as a methyl ester. This alternative is shown in Scheme 2. Subsequently, the alkyl ester group is hydrolyzed to a carboxylic acid and the acyl groups protecting the phenolic hydroxyls are removed. The chicoric acid analogue or derivative thus produced has one carboxyl group in its central linker.

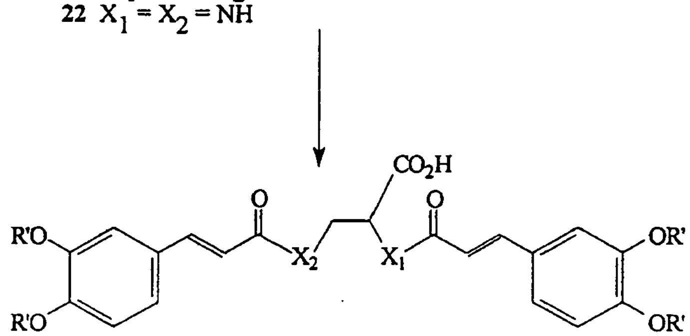
Scheme 2:





for R = AC
18 $X_1 = X_2 = O$
19 – 21 $X_1 = NH, X_2 = O$
22 $X_1 = X_2 = NH$

for R = Me
23 $X_1 = X_2 = NH$



for R' = AC
24 $X_1 = X_2 = O$
25 $X_1 = X_2 = NH$

for R' = Me
26 $X_1 = X_2 = NH$

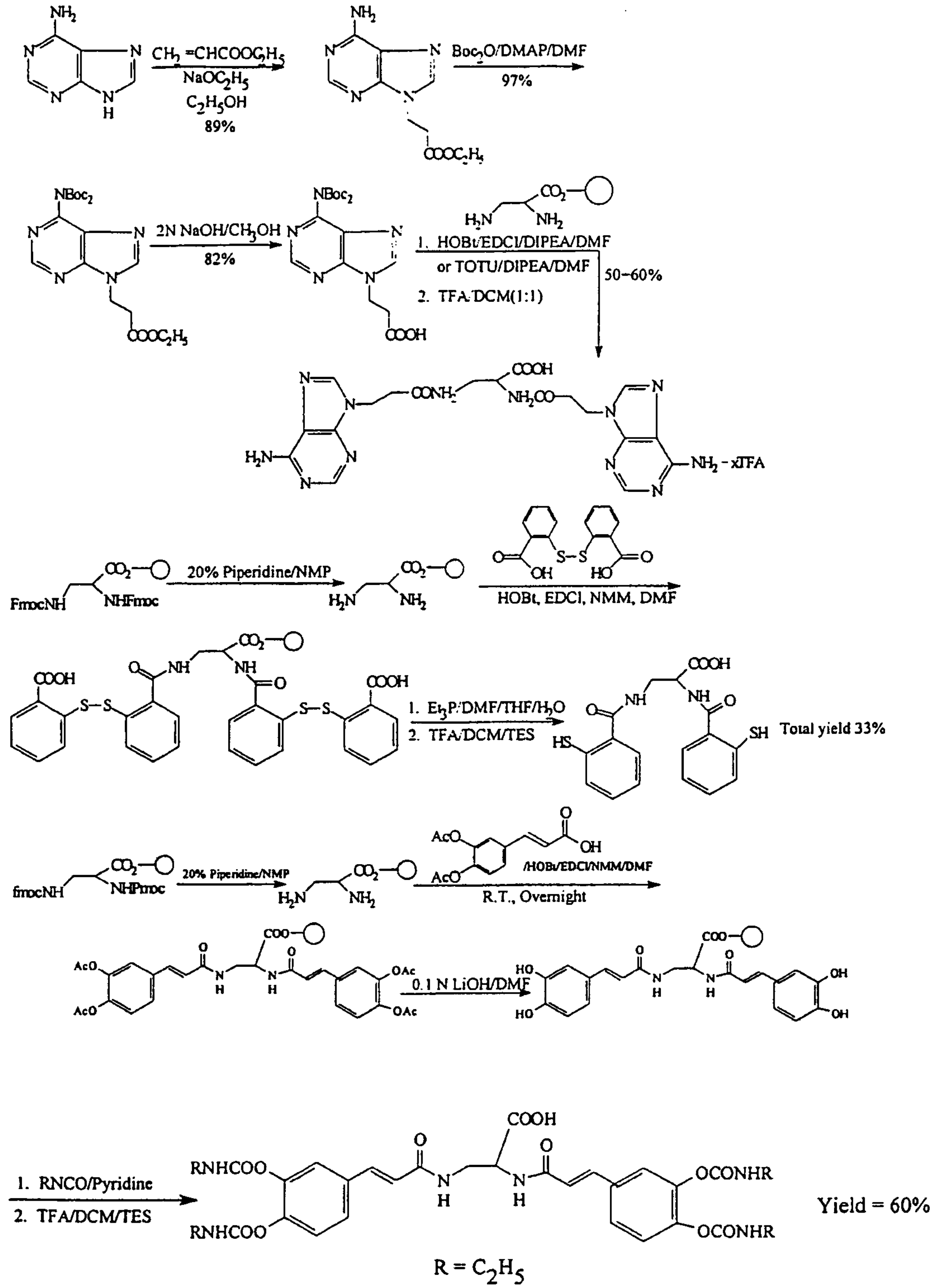
for R' = H
27 $X_1 = X_2 = O$
28 $X_1 = NH, X_2 = O$

Yet another method of synthesis of chicoric acid analogues and derivatives according to the present invention involves combinatorial solid–phase organic synthesis. N.K. Terrett et al., "Combinatorial Synthesis: The Design of Compound Libraries and Their Application to Drug Discovery," Tetrahedron 51: 8135–8173 (1995); R.F. Service, "Combinatorial Chemistry Hits the Drug Market," Science 272 1266–1268 (1996).

The chicoric acid analogues and derivatives of the present invention are examples of compounds that have the generalized structure of "Pharmacophore A" (Figure 1). In this model, these compounds are composed of three components, consisting of two aryl units and a central linker. Suitable solid–phase supports are well known in the art J.S. Fruchtel & G. Jung, "Organic Chemistry on Solid Supports," Angew. Chem. Int. Ed. Engl. 35: 17–42 (1996). Other solid–phase combinatorial methods are known in the art and are described, for example, in I. Sucholeiki, "Solid–Phase Methods in Combinatorial Chemistry," in Combinatorial Chemistry: Synthesis and Application (S.R. Wilson & A.W. Czarnyk, eds., John Wiley & Sons, Inc., New York, 1997), pp. 119–133.

A particular example of a solid–phase synthetic scheme for synthesis of a chicoric acid analogue is shown in Scheme 3. In Scheme 3, Boc_2O is butyloxycarbonyl anhydride. DMAP is dimethylaminopyridine. DMF is dimethylformamide. HOBt is hydroxybenzotriazole. EDCI is 1–(3–dimethylaminopropyl)–3–ethylcarbodiimide hydrochloride. DIPEA is diisopropylethylamine. TOTU is O–[(ethoxycarbonyl)cyanomethyleneamino]–N,N,N′,N′–tetramethyluronium tetrafluoroborate. TFA is trifluoroacetic acid. DCM is dichloromethane. NMP is N–methylpyrrolidinone. NMM is N–methylmorpholine. Et_3P is triethyl phosphine. TES is tetraethylsilane. Fmoc is 9–fluorenylmethyloxycarbonyl.

Scheme 3:



A method of synthesis of an example of the generic compound described above as Formula (**I**), where X_1 and X_2 are both NH, Z_1 is H, Z_2 is CO_2H, and each of Y_1, Y_2, Y_3, and Y_4 is O, and each of R_1, R_2, R_3, and R_4 is a moiety forming an ethylcarbamate, is shown in Scheme 3.

III. USE OF CHICORIC ACID ANALOGUES AND DERIVATIVES ACCORDING TO THE PRESENT INVENTION

Chicoric acid analogues and derivatives according to the present invention can be used in *in vivo* methods for inhibiting the replication of HIV−1. They can also be used in *ex vivo* and *in vitro* methods for inhibiting the replication of HIV−1, such as in screening methods to determine the relative susceptibility of strains of HIV−1 to integrase inhibitors and to identify resistant or possibly resistant strains to determine the appropriate course of therapy. This is discussed below.

A. *In Vivo* Methods for Inhibiting the Replication of HIV−1

Chicoric acid analogues and derivatives according to the present invention can be used in *in vivo* methods for inhibiting the replication of HIV−1, either alone or in combination with other drugs such as reverse transcriptase inhibitors or protease inhibitors.

Chicoric acid analogues and derivatives suitable for *in vivo* use have the following properties: (1) efficient *in vitro* inhibition of the integrase, as measured by the 3′−processing and strand transfer reactions carried out by the integrase; (2) ability to prevent replication of the HIV−1 virus in a cytoprotection assay to measure the ability of compounds to protect host cells from HIV−1 from the cytopathic effects of a virus strain; and (3) relatively low cytotoxicity on uninfected cells. Methods for assessing these properties are set forth in the Examples, namely in Example 2.

In general, these methods comprise administering to a patient infected with HIV−1 a compound in a quantity sufficient to inhibit the replication of HIV−1 by detectably inhibiting the activity of HIV−1 integrase in the patient.

The compounds suitable for such in vivo methods include the novel compounds described above, with the addition of D−(+)−chicoric acid. Therefore, these compounds are described by formula (I), above, where:

Q is a valence bond or CH_2;

X_1 is O, NH, or CH_2;

X_2 is O, NH, or CH_2;

Y_1, Y_2, Y_3, and Y_4 are each a valence bond, O, or NH;

where Y_1, Y_2, Y_3, or Y_4 is a valence bond, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is a carboxy−containing moiety selected from the group consisting of carboxymethyl, carboxyethyl, carboxypropyl, carboxy small alkyl and carboxy aryl;

where Y_1, Y_2, Y_3, or Y_4 is O, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each H, acetyl, propionyl, butyryl, or isobutyryl, or is a moiety forming a lower alkyl carbamate or an aryl carbamate;

where Y_1, Y_2, Y_3, or Y_4 is NH, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each acetyl, propionyl, butyryl, isobutyryl, small alkyl or aryl;

Z_1 and Z_2 are each H, lower alkyl, −CHO, $-CO_2H$, or $-CO_2W$, where W is lower alkyl or aryl, or, alternatively, where Q is a valence bond, Z_1 and Z_2, together with the adjacent carbon atoms and Q, form a ring structure, the carbon skeleton of the ring structure being selected from the group consisting of cyclohexane, cyclohexene, cyclopentane, cycloheptane, cycloheptene, and benzene;

with the proviso that where each of Y_1, Y_2, Y_3, or Y_4 is O and all of R_1, R_2, R_3, and R_4 are other than H, at least one of Z_1 or Z_2 is $-CO_2H$ or $-CO_2W$;

and with the proviso that either: (1) where X_1 and X_2 are both O, Q is a valence bond, and Z_1 and Z_2 are both $-CO_2H$, either at least one of R_1, R_2, R_3, and R_4 is other than H or at least one of Y_1, Y_2, Y_3, and Y_4 is other than O, or, (2) that where X_1 and X_2 are both O, Q is a valence bond, Z_1 and Z_2 are both $-CO_2H$, all of R_1, R_2, R_3, and R_4 are H, and all of Y_1, Y_2, Y_3, and Y_4 are O, the compound is the D−(+) enantiomer.

Although chicoric acid analogues and derivatives according to the present invention can be used alone for *in vivo* treatment, it is generally preferred to administer them along with other agents capable of *in vivo* inhibition or blockage of replication of HIV−1. These agents include protease inhibitors and reverse transcriptase inhibitors. The use of these agents is well known in the art and need not be described further herein. Reverse transcriptase inhibitors include zidovudine

(3′–azido–3′–deoxythymidine), didanosine (2′,3′–dideoxyinosine), stavudine (2′,3′–didehydro–2′,3′–dideoxythymidine), zalcitabine (2′,3′–dideoxycytidine), lamivudine, the (–) enantiomer of 2′–deoxy–3′–thiacytidine, FTC (2′,3′–dideoxy–5–fluoro–3′–thiacytidine), adefovir, nevirapine, delaviridine, loviride, and other agents. Protease inhibitors include saquinavir, indinavir, and other agents.

The appropriate dosages and routes of administration of these agents can be determined by the treating physician based upon such consideration as the clinical status of the patient, the weight, size, and age of the patient, the T–cell count of the patient, the particular strain of HIV–1 infecting the patient, the presence or absence of opportunistic infections, the use of other therapies, the response of the patient to the therapies, and pharmacokinetic considerations such as kidney and liver function, which can affect the metabolism and excretion of the agents being administered.

Typically, the chicoric acid analogues and derivatives, together with other antiviral agents if used, are administered in a conventional pharmaceutically acceptable formulations, preferably including a carrier. Conventional pharmaceutically acceptable carriers known in the art can include alcohols, e.g., ethyl alcohol, serum proteins, human serum albumin, liposomes, buffers such as phosphates, water, sterile saline or other salts, electrolytes, glycerol, hydroxymethylcellulose, propylene glycol, polyethylene glycol, polyoxyethylenesorbitan, other surface active agents, vegetable oils, and conventional anti–bacterial or anti–fungal agents, such as parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. A pharmaceutically–acceptable carrier within the scope of the present invention meets industry standards for sterility, isotonicity, stability, and non–pyrogenicity. The particular carrier used depends on the concentration of the agent capable of blocking the immune defense suppressive effect and the intended route of administration. Typically, a pharmaceutical composition according to the present invention is injected by one of a number of conventional routes, such as intravenous, intradermal, intraperitoneal, or intramuscular, or is administered parenterally.

B. *Ex Vivo* and *In Vitro* Methods for Inhibiting HIV−1 Integrase

Another aspect of the present invention is *ex vivo* and *in vitro* methods for inhibiting HIV−1 integrase or protecting susceptible target cells from HIV−1 infection. These methods are based on the assays described in the Examples for inhibition of the 3′−processing and strand transfer reactions of the integrase, and for cytoprotection to determine the ability of these compounds to inhibit the replication of HIV−1 in susceptible target cells and thus to protect these cells.

In general, such methods comprise contacting HIV−1 integrase with a quantity of a compound sufficient to produce a detectable inhibition of HIV−1 integrase. The compounds are the novel compounds described above, with the addition of D−(+)−chicoric acid. Therefore, these compounds are described by formula (I), above, where:

Q is a valence bond or CH_2;

X_1 is O, NH, or CH_2;

X_2 is O, NH, or CH_2;

Y_1, Y_2, Y_3, and Y_4 are each a valence bond, O, or NH;

where Y_1, Y_2, Y_3, or Y_4 is a valence bond, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is a carboxy−containing moiety selected from the group consisting of carboxymethyl, carboxyethyl, carboxypropyl, carboxy small alkyl and carboxy aryl;

where Y_1, Y_2, Y_3, or Y_4 is O, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each H, acetyl, propionyl, butyryl, or isobutyryl, or is a moiety forming a lower alkyl carbamate or an aryl carbamate;

where Y_1, Y_2, Y_3, or Y_4 is NH, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each acetyl, propionyl, butyryl, isobutyryl, small alkyl or aryl;

Z_1 and Z_2 are each H, lower alkyl, −CHO, $-CO_2H$, or $-CO_2W$, where W is lower alkyl or aryl, or, alternatively, where Q is a valence bond, Z_1 and Z_2, together with the adjacent carbon atoms and Q, form a ring structure, the carbon skeleton of the ring structure being selected from the group consisting of cyclohexane, cyclohexene, cyclopentane, cycloheptane, cycloheptene, and benzene;

with the proviso that where each of Y_1, Y_2, Y_3, or Y_4 is O and all of R_1, R_2, R_3, and R_4 are other than H, at least one of Z_1 or Z_2 is $-CO_2H$ or $-CO_2W$;

and with the proviso that either: (1) where X_1 and X_2 are both O, Q is a valence bond, and Z_1 and Z_2 are both $-CO_2H$, either at least one of R_1, R_2, R_3, and R_4 is other than H or at least one of Y_1, Y_2, Y_3, and Y_4 is other than O, or, (2) that where X_1 and X_2 are both O, Q is a valence bond, Z_1 and Z_2 are both $-CO_2H$, all of R_1, R_2, R_3, and R_4 are H, and all of Y_1, Y_2, Y_3, and Y_4 are O, the compound is the D−(+) enantiomer.

These methods are useful in screening HIV−1 isolates for their susceptibility to reverse transcriptase inhibitors and thus to enable treating physicians to detect the emergence or possible emergence of resistant strains of HIV−1. This will enable treating physicians to adjust drug therapies appropriately.

IV. COMPOSITIONS COMPRISING CHICORIC ACID ANALOGUES AND DERIVATIVES

Another aspect of the present invention is compositions comprising chicoric acid analogues and derivatives. These compositions typically comprise: (1) a chicoric acid analogue or derivative in a quantity sufficient to detectably inhibit at least one reaction of integrase selected from the group consisting of 3′−processing and strand transfer; and (2) an acceptable carrier.

Conventional acceptable carriers known in the art can include alcohols, e.g., ethyl alcohol, serum proteins, human serum albumin, liposomes, buffers such as phosphates, water, sterile saline or other salts, electrolytes, glycerol, hydroxymethylcellulose, propylene glycol, polyethylene glycol, polyoxyethylenesorbitan, other surface active agents, vegetable oils, and conventional anti−bacterial or anti−fungal agents, such as parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. An acceptable carrier within the scope of the present invention meets industry standards for sterility, isotonicity, stability, and non−pyrogenicity.

The composition can also be in capsule, tablet, or lozenge form as is known in the art, and can include excipients or other ingredients for greater stability or acceptability. For the tablets, the excipients can be inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium

stearate, stearic acid, or talc, along with the chicoric acid analogue or derivative and other ingredients.

The compounds included in the compositions are the novel chicoric acid analogues and derivatives described above. These compounds are those of formula (I) where:

Q is a valence bond or CH_2;

X_1 is O, NH, or CH_2;

X_2 is O, NH, or CH_2;

Y_1, Y_2, Y_3, and Y_4 are each a valence bond, O, or NH;

where Y_1, Y_2, Y_3, or Y_4 is a valence bond, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is a carboxy−containing moiety selected from the group consisting of carboxymethyl, carboxyethyl, carboxypropyl, carboxy small alkyl and carboxy aryl;

where Y_1, Y_2, Y_3, or Y_4 is O, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each H, acetyl, propionyl, butyryl, or isobutyryl, or is a moiety forming a lower alkyl carbamate, or an aryl carbamate;

where Y_1, Y_2, Y_3, or Y_4 is NH, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each acetyl, propionyl, butyryl, isobutyryl, small alkyl or aryl;

Z_1 and Z_2 are each H, lower alkyl, $-CHO$, $-CO_2H$, or $-CO_2W$, where W is lower alkyl or aryl, or, alternatively, where Q is a valence bond, Z_1 and Z_2, together with the adjacent carbon atoms and Q, form a ring structure, the carbon skeleton of the ring structure being selected from the group consisting of cyclohexane, cyclohexene, cyclopentane, cycloheptane, cycloheptene, and benzene;

with the proviso that where each of Y_1, Y_2, Y_3, or Y_4 is O and all of R_1, R_2, R_3, and R_4 are other than H, at least one of Z_1 or Z_2 is $-CO_2H$ or $-CO_2W$;

and with the proviso that where X_1 and X_2 are both O, Q is a valence bond, and Z_1 and Z_2 are both $-CO_2H$, either at least one of R_1, R_2, R_3, and R_4 is other than H or at least one of Y_1, Y_2, Y_3, and Y_4 is other than O.

The following Examples illustrate the advantages of the subject invention. These Examples are for illustrative purposes only and are not to be construed as limiting the scope of the invention in any manner.

EXAMPLES

All formula designations in the following examples refer to the compounds found in Table 1 of this specification.

Example 1

Synthesis of Chicoric Acid Analogues and Derivatives

L-(-)-chicoric acid (formula D) and D-(+)-chicoric acid (formula 4) were prepared from di-*tert*-butyl esters of L-(+)-tartaric acid and D-(-)-tartaric acid respectively, using 3,4-diacetylcaffeoyl acid chloride (formula 5) as previously reported. Zhao et al., "Facile synthesis of (2R,3R)-(-)-and (2S,3S)-(+)-chicoric acids," Synthetic Commun. 28, 737-740 (1998). The tetra-acetylated L-chicoric acid (formula 6) and D-chicoric acid (formula 7) were similarly prepared. Zhao et al, Synthetic Commun., supra. Tetra-acetylated bis-caffeoyl derivatives of formulas 8 - 12 were also synthesized by treatment of the corresponding diols or amino alcohol with acid chloride (formula 5) in the presence of pyridine. Hydrolysis of the acetates to yield the free bis-caffeoyl analogues of formulas 13 - 17 was achieved by refluxing the tetra-acetates in acetone containing aqueous HCl (Scheme 1).

Monocarboxy analogues of formulas 18 - 29 were prepared in a similar, but slightly altered fashion (Scheme 2). Central linkers were first esterified as their methyl esters (refluxing methanolic HCl), then acylated with either diacetylcaffeoyl chloride (formula 5) in pyridine (for formulas 18, 19 - 21) or with diacetylcaffeic acid via hydroxybenzotriazole (HOBt) mixed anhydride coupling (for formulas 22 and 23). Using lithium iodide in refluxing pyridine, methyl esters of formulas 18, 22, 23 were then selectively cleaved in the presence of acetoxy and methyl ether functionality, to afford free acids of formulas 24 - 26, which retained caffeoyl protection. Alternatively, refluxing methyl esters of formulas 18 and 19 in acetone containing aqueous HCl provided globally deprotected free acids of formulas 27 and 28 respectively, without cleavage of the caffeoyl linkages (formula 29 shown in Table 1).

These syntheses are set forth in detail below. For these syntheses, melting points were determined on either a Gallenkamp or a Mell Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA, and are within 0.4% of the theoretical values unless otherwise indicated. Fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. ^{1}H NMR data were obtained on a Bruker AC250 spectrometer (250 MHz) and are reported in ppm relative to TMS and referenced to the solvent in which they were run. Anhydrous solvents were obtained commercially and used without further drying. Flash column chromatography was performed using E. Merck silica gel 60 (particle size, 230-400 mesh). HPLC was done on a Waters PrepLC 4000 System using Vydac C18 peptide/protein analytical column. Optical rotation was taken on a Perkin-Elmer 241 digital polarimeter at ambient temperature.

General method for the preparation of formulas 8 - 12. To a solution of commercially available diol or ethanolamine (1 mmol) in anhydrous pyridine (3 mL) was added dropwise under argon a solution of freshly prepared 3,4-diacetyl caffeoyl acid chloride (Zhao et al, Synthetic Commun., supra.) formula 5 (2.5 mmol) in toluene (5 mL). The mixture was stirred at room temperature (overnight), then pyridine was removed under reduced pressure. The residue was taken to dryness from toluene (three times) then purified by silica gel chromatography and crystallized to provide the desired product.

(+)-(2S,3S)-O,O-Bis-(3,4-diacetoxycinnamoyl)-2,3-butanediol (formula 8). Reaction of (2S,3S)-butanediol as described above provided formula 8 as a foam (92% yield). $[\alpha]^{20}{}_D + 54°$ (MeOH, c = 0.14); ^{1}H NMR ($CDCl_3$) δ 7.6 (d, J = 16.0 Hz, 2H), 7.39-7.35 (m, 4H), 7.19 (d, J = 8.2 Hz, 2H), 6.37 (d, J = 16.0 Hz, 2H), 5.14 (dq, J = 6.0, 3.8 Hz, 2H), 2.28 (s, 3H), 2.27 (s, 3H), 1.3 (d, J = 6.0 Hz, 6H). Anal. ($C_{30}H_{30}O_{12}$•H_2O) C, H.

(+) - (1S,2S)-O,O-Bis-(3,4-diacetoxycinnamoyl)-1,2-cyclohexanediol (formula 9). Reaction of (1S,2S)-cyclohexanediol as described above provided formula 9 as a solid (91% yield); mp 62-64°C (EtOAc-hexane). $[\alpha]^{20}{}_{D}$ + 220° (MeOH, c = 0.13); ^{1}H NMR ($CDCl_3$) δ 7.55 (d, J = 16.0 Hz, 2H), 7.36-7.3 (m, 4H), 7.17 (d, J = 8.3 Hz, 2H), 6.3 (d, J = 16.0 Hz, 2H), 5.0-4.97 (m, 2H), 2.27 (s, 3H), 2.26 (s, 3H), 2.16-2.11 (m, 2H), 1.78 (m, 2H), 1.47-1.4 (m, 4H). Anal. ($C_{32}H_{32}O_{12}$•H_2O) C, H.

O,O-Bis-(3,4-diacetoxycinnamoyl)-1,2-ethanediol (formula 10). Reaction of 1,2-ethanediol as described above provided formula 10 as a solid (89% yield; mp 153-156°C (EtOAc-hexane). ^{1}H NMR (DMSO-d_6) δ 7.72-7.64 (m, 6H), 7.31 (d, J = 8.4 Hz, 2H), 6.7 (d, J = 16.1 Hz, 1H), 4.43 (s, 4H). Anal. ($C_{28}H_{26}O_{12}$) C, H.

O,O-Bis-(3,4-diacetoxycinnamoyl)-1,3-propenediol (formula 11). Reaction of 1,3-propenediol as described above provided formula 11 as a solid (91% yield); mp 194.5-196°C (EtOAc-hexane). ^{1}H NMR (DMSO-d_6) δ 7.7-7.62 (m, 6H), 6.09 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 16.1 Hz, 1H), 4.47 (t, J = 6.2 Hz, 4H), 2.283 (s, 6H), 2.04 (quintuplet, J = 6.3 hZ, 2h) Anal. ($C_{29}H_{28}O_{12}$•H_2O) C, H.

N,O-Bis-(3,4-diacetoxycinnamoyl)-2-hydroxyethylamine (formula 12). Reaction of 2-hydroxyethylamine as described above provided formula 12 as a solid (96% yield); mp 177-179.5°C (EtOAc-hexane). ^{1}H NMR ($CDCl_3$) δ 7.66 (d, J = 16.1 Hz, 1H), 7.57 (d, J = 15.6 Hz, 1H), 7.42-7.32 (m, 4H), 7.21 (d, J = 8.5 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.38 (d, J = 16.0 Hz, 1H), 6.34 (d, J = 15.4 Hz, 1H), 6.00 (t, J = 5.3 Hz, 1H), 4.36 (t, J = 5.1 Hz, 2H), 3.71 (dt, J = 5.1, 5.3 Hz, 2H), 2.29 (s, 3H), 2.285 (s, 3H), 2.280 (s, 3H), 2.276 (s, 3H). Anal. ($C_{28}H_{21}NO_{11}$) C, H, N.

General method for preparation of compounds formulas 13 - 17. Diesters of formulas 8 - 11 or amide-ester formula 12 (0.5 mmol) in acetone (15 mL) were refluxed (3 h) with 3N HCl (5 mL), then cooled to room temperature, diluted with EtOAc (100 mL) and washed with brine. After drying (Na_2SO_4) samples were

concentrated and purified by either crystallization or HPLC to give the desired products.

(+)-(2S,3S)-O,O-Bis-(3,4-dihydroxycinnamoyl)-2,3-butanediol (formula 13). Treatment of the diester of formula 8 as described above and purification by HPLC (linear gradient, MeOH in H_2O from 0% to 100% MeOH over 30 min) provided formula 13 as a white solid (73% yield). $[\alpha]^{20}{}_D$ + 98° (MeOH, c = 0. 13); ^{1}H NMR (DMSO-d_6) δ 7.47 (d, *J* = 16.0 Hz, 2H), 7.03-6.96 (m, 4H), 6.73 (d, *J* = 8.1 Hz, 2H), 6.24 (d, *J* = 15.9 Hz, 2H), 5.05 (dq, *J* = 6.0, 3.8 Hz, 2H), 1.21 (d, *J* = 6.0 Hz, 6H); FABMS *m/z* 413 (M-H). Anal. ($C_{22}H_{22}O_8$ •H_20) C, H.

(+)-(1S,2S)-O,O-Bis-(3,4-dihydroxycinnamoyl)-1,2-cyclohexanediol (formula 14). Treatment of the diester of formula 9 as described above provided formula 14 as a solid (88% yield); mp 216-218° C (EtOAc-$CHCl_3$). $[a]^{20}{}_D$ +373° (MeOH, c = 0.18); ^{1}H NMR (DMSO-d_6)δ 7.42 (d, *J* = 15.9 Hz, 2H), 6.98-6.92 (m, 4H), 6.7 (d, *J* = 8.1 Hz 2H), 6.16 (d, *J* =15.9 Hz, 2H), 4.9 (m, 2H), 1.98 (m, 2H), 1.68 (m, 2H), 1.4 (m, 4H); FABMS *m/z* 439 (M-H). Anal. ($C_{24}H_{24}O_8$•1¼ H_2O) C, H.

O,O-Bis-(3,4-dihydroxycinnamoyl)-1,2-ethanediol (formula 15). Treatment of the diester of formula 10 as describe above provided formula 15 as a solid (89% yield); mp> 330°C (MeOH-$CHCl_3$) ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 2H), 9.13 (s, 2H), 7.5 (d, *J* = 15.9 Hz, 2H), 7.04-6.99 (m, 4H), 6.75 (d, *J* = 8.1 Hz, 2H), 6.29 (d, *J* = 15.9 Hz 1H), 4.37 (s, 4H); FABMS *m/z* 385 (M-H). Anal. ($C_{20}H_{18}O_8$•H_2O) C, H.

O,O-Bis-(3,4-dihydroxycinnamoyl)-1,3-propenediol (formula 16). Treatment of the diester of formula 11 as described above provided formula 16 as a solid (83% yield); mp 205-207°C (MeOH-$CHC1_3$). ^{1}H NMR (DMSO-d_6) δ 7.48 (d, *J* = 5.9 Hz, 2H), 7.03 (d, *J* = 1.8 Hz, 2H), 6.97 (dd, *J* = 8.2, 1.8 Hz, 2H), 6.68 (d, *J* = 8.1 Hz, 2H), 6.24 (d, *J*=-15.9 Hz, 2H), 4.21 (t, *J* = 6.2 Hz, 4H), 2.0 (t, *J* = 6.1 Hz, 2H); FABMS *m/z* 399 (M-H). Anal. ($C_{21}H_{20}O_8$•H_2O) C, H.

N,O-Bis-(3,4-dihydroxycinnamoyl)-2-hydroxyethylamine (formula 17). Treatment of the diester of formula 12 as described above provided formula 17 as a solid (88% yield); mp 214-215.5°C (MeOH-$CHCl_3$). ^{1}H NMR ($DMSO-d_6$) δ 9.59 (s, 1H), 9.35 (s, 1H), 9.14 (s, 1H), 9.11 (s, 1H), 8.17 (t, *J* = 5.4 Hz, 1H), 7.5 (d, *J* = 15.9 Hz, 1H), 7.25 (d, *J* = 15.7 Hz, 1H), 7.04-6.93 (m, 2H), 6.85-6.71 (m, 2H), 6.34 (d, *J* = 15.7 Hz, 1H), 6.25 (d, *J* = 15.5 Hz, I H), 4.16 (t, *J* = 5.4 Hz, 2H), 3.45 (m, 2H); FABMS *m/z* 384 (M-H). Anal. ($C_{20}H_{19}N_7$•0.5 H_2O) C, H, N.

Methyl O,O-bis-(3,4-diacetoxycinnamoyl)-2,3-dihydroxypropanoate (formula 18). To a solution of 2,3-dihydroxypropanoic acid methyl ester (5.0 mmol) in anhydrous pyridine (20 mL) was added a solution of 3,4-diacetyl caffeoyl acid chloride formula 5 (12.5 mmol) in toluene (30 mL) and the resulting cloudy solution was stirred at room temperature overnight. Solvent was removed by rotary evaporator and residue purified by flash chromatography (ether:$CHCl_3$) to afford formula 18 as a pale white solid (2.70 g, 88% yield); mp 159 - 160°C. ^{1}H NMR ($DMSO-d_6$) δ 7.80 (d, *J* = 16.1 Hz, I H), 7.73 (d, *J* = 16.2 Hz, 1H), 7.30-7.48 (m, 6H), 6.57 (d, *J* =16.1 Hz, 1H), 6.48 (d, *J* = 16.1 Hz, 1H), 5.60 (t, *J* = 4.9 Hz, 1H), 4.74 (m, 2H), 3.91 (s, 3H), 2.39 (s, 12H); FABMS *m/z* 613 (M+H). Anal.($C_{30}H_{28}O_{14}$) C, H.

Methyl N,O-bis-(3,4-diacetoxycinnamoyl)serinate (formula 19). Using racemic methyl serinate hydrochloride in a procedure similar to that described above for the synthesis of formula 18, the product of formula 19 was obtained as a white solid (84% yield); mp 105.5-107°C. ^{1}H NMR ($CDCl_3$) δ 7.70 (d, *J* = 16.0 Hz, 1H), 7.67 (d, *J* = 15.6 Hz, 1H), 7.29-7.40 (m, 6H), 6.55 (d, *J* = 8.0 Hz, 1H), 6.50 (d, *J* = 15.6 Hz, IH), 6.44 (d, *J* = 16.1 Hz, 1H), 5.11 (m, 1H), 4.69 (q, *J* = 3.4 Hz, 2H), 3.90 (s, 3H), 2.38 (s, 12H); FABMS *m/z* 612 (M+H). Anal. ($C_{30}H_{29}NO_{13}$) C, H, N.

Methyl N,O-bis-(3,4-diacetoxycinnamoyl)-L-serinate (formula 20). Using methyl L-serinate hydrochloride in a procedure similar to that described

above for the synthesis of formula 18, the product of formula 20 was obtained as a white powder (76% yield). NMR spectral data was the same as that reported above for formula 19. $[\alpha]^{20}{}_{D}$ - 105.5° ($CHCl_3$, c = 0.56). Anal. ($C_{30}H_{29}NO_{13}$•0.5 H_2O) C, H, N.

Methyl N,O-bis-(3,4-diacetoxycinnamoyl)-D-serinate (formula 21). Using methyl D-serinate hydrochloride in a procedure similar to that described above for the synthesis of formula 18, the product of formula 21 was obtained as a white powder (81% yield). NMR spectral data was the same as that reported above for formula 19. $[a]^{20}{}_{D}$ -108.6° ($CHCl_3$, c = 0.63). Anal. ($C_{30}H_{29}NO_{13}$•0.5 H_2O) C, H, N.

Methyl N,N-bis-(3,4-diacetoxycinnamoyl)-2,3-diaminopropanoate (formula 22). To a mixture of N-methylmorpholine (52.1 mmol), methyl 2,3-diaminopropanoate dihydrochloride (5.23 mmol) 3,4-diacetoxycinnamic acid (10.5 mmol), and 1-hydroxybenzotriazole hydrate (10.5 mmol) in CH_2Cl_2 (30 mL) was added diisopropylcarbodiimide (13.60 mmol). The resulting solution was stirred at room temperature (overnight) then partitioned between CH_2Cl_2 (~200 mL) and H_2O (~70 mL), washed with brine and dried (Na_2SO_4). Solvent was removed by rotary evaporator and residue purified by flash column chromatography ($CHCl_3$ and $CHCl_3$:EtOAc) to afford formula 22 as a white crystalline solid (1.40 g, 44% yield); mp, 151-152 °C. ^{1}H NMR (DMSO-d_6) δ 7.65 (d, J = 15.8 Hz, 1H), 7.64 (d, J = 15.6 Hz, 1H), 7.26-7.34 (m, 6H), 6.54 (br s, 2H), 6.5 1 (d, J = 15.8 Hz, 1H), 6.41 (d, J = 15.6 Hz, 1H), 4.85 (q, J = 4.6 Hz, 1H), 3.92 (d, J = 4.6 Hz, 2H), 3.89 (s, 3H), 2.39 (s, 6H), 2.38 (s, 6H); FABMS *m/z* 611 (M+H). Anal. ($C_{30}H_{30}N_2O_{12}$) C, H, N.

Methyl N,N -bis-(3,4- methoxycinnamoyl)-2,3 -diaminopropanoate (formula 23). Using 3,4-dimethoxycinnamic acid in a procedure similar to that described above for the synthesis of formula 22, the product of formula 23 was obtained as a pale solid (67% yield); mp 125 - 126°C. ^{1}H NMR (DMSO-d_6) δ 7.65 (d, J = 15.6 H, 1H), 7.64 (d, J = 15.5 Hz, 1H), 7.23 (d, J = 6.8 Hz, I H), 6.90-7.13 (m, 6H), 6.62 (t, J = 6.7 Hz, 1H), 6.45 (d, J=15.6 Hz, lH),

6.37 (d, *J* 15.5 Hz, 1H), 4.90 (q, *J* 6.6 Hz, 1H), 3.98 (s, 12H), 3.92 (m, 2H), 3.88 (s, 3H); FABMS *m/z* 499 (M+H). Anal. ($C_{26}H_{30}N_2O_8$) C, H, N.

General procedure for demethylation of compounds formulas 18, 22 and 23 to provide formulas 24 - 26, respectively. A suspension of methyl ester (0.4 mmol) and lithium iodide (3.2 mmol) in pyridine (3 mL) was stirred at reflux (1 h). Pyridine was removed and the residue was taken up in H_2O (30 mL), acidified with 6 N HCl (to pH 3), extracted with EtOAc (2 x 50 mL), washed with brine and dried (Na_2SO_4) . Removal of solvent provided products as solids.

O,O-Bis-(3,4-diacetoxycinnamoyl)-2,3-dihydroxypropanoic acid (formula 24). Treatment of methyl ester 18 as described above in the general procedure provided formula 24 as a white solid (63% yield); mp: 130 °C (dec) ^{1}H NMR (DMSO-d_6) δ 13.50 (br s, 1H), 7.88 (m, 2H), 7.38- 7.80 (m, 6H), 6.81 (d, *J* = 16.4 Hz, 1H), 6.74 ,(d, *J* = 16.1 Hz, 1H), 5.48 (t, *J* = 3.5 Hz, 1H), 4.65 (d, *J* = 3.5 Hz, 2H), 2.34 (br s, 12H); FABMS *m/z* 597 (M-H). Anal. ($C_{29}H_{26}O_{14}$•$2.5H_2O$) C, H.

N,N-Bis-(3,4-diacetoxycinnamoyl)-2,3-diaminopropanoic acid (formula 25). Treatment of methyl ester formula 22 as described above in the general procedure provided formula 25 as a white solid (67% yield); mp 120°C (dec). ^{1}H NMR (DMSO-d_6) δ 12.90 (br s, 1H), 10.15 (br d, 1H), 9.94 (br d, 1H), 8.47 (m, 2H), 7.06-7.54 (m, 6H) 6.69 (m, 2H), 4.55 (m, 1H), 3.70 (br s, 2H), 2.34 (s, 12H); FABMS *m/z* 595 (M-H). Anal. ($C_{29}H_{28}N_2O_{12}$•0.4 C_5H_5N) C, H, N.

N,N-Bis-(3,4-dimethoxycinnamoyl)-2,3-diaminopropanoic acid (formula 26). Treatment of methyl ester formula 23 as described above in the general procedure provided formula 26 (84 % yield); mp 108°C (dec). ^{1}H NMR (DMSO-d_6) δ 12.90 (br s, 1H), 8.30 (d, *J* = 7.8 Hz, 1H), 8.16 (t, *J* = 5.9 Hz, 1H), 7.42 (d, *J* = 15.9 Hz, 1H), 7.41 (d, *J* = 15.6 Hz, 1H), 7.02-7.19 (m, 6H), 6.66 (d, *J* = 15.9 Hz, 1H), 6.58 (d, *J* = 15.6 Hz, 1H), 4.52 (m, 1H), 3.84 (s, 3H), 3.83 (s, 6H), 3.82 (s, 3H), 3.52-3.68 (m, 2H); FABMS *m/z* 483 (M-H). Anal. ($C_{25}H_{28}N_2O_8$) C, H, N.

O,O-Bis-(3,4-dihydroxycinnamoyl)-2,3-dihydroxypropanoic acid (formula 27). A suspension of formula 18 in a mixture of 3 N HCl: acetone (v/v 1:3) was refluxed (3 h) then cooled to room temperature and diluted with an equal volume of H_2O and extracted with EtOAc. Combined organic extracts were dried (Na_2SO_4), taken to dryness and residue purified by silica gel flash chromatography (EtOAc/AcOH) to provide formula 27 as a white solid (30% yield); mp 85-90°C. ^{1}H NMR (DMSO-d_6) δ 11.15 (br s, 1H), 8.45 (br s, 4H), 7.69 (d, J = 15.9 Hz, 1H), 7.64 (d, J = 15.9 Hz, 1H), 6.92-7.24 (m, 6H), 6.40 (m, 2H), 5.56 (dd, J = 3.2, 8.6 Hz, 1H), 4.72 (m., 2H); FABMS *m/z* 429 (M-H). Anal. ($C_{21}H_{18}O_{10}$•0.6 H_2O) C, H.

N,O-Bis-(3,4.dihydroxycinnamoyl)serine (formula 28). Treatment of formula 19 as described above for the conversion of formulas 18 to 27, provided formula 28 as a white solid (71% yield); mp 112°C (dec). ^{1}H NMR (DMSO-d_6) δ 13.05 (br s, 1H), 9.67 (s, 1H), 9.44 (s, 1H), 9.21 (s, 1H), 9.19 (s, 1H), 8.49 (d, J = 8.0 Hz, 1H), 7.55 (d, J 15.9 Hz, 1H), 7.32 (d, J = 15.6 Hz, 1H), 6.80-7.04 (m, 6H), 6.55 (d, J = 15.6 Hz, 1H), 6.27 (d, J = 15.9 Hz, 1H), 4.78 (m, 1H), 4.44 (d, J = 4.6 Hz, 2H); FABMS *m/z* 428 (M-H). Anal. ($C_{21}H_{19}NO_9$•0.7 H_2O) C, H, N.

Methyl N-acetyl-O-(3,4-diacetoxycinnamoyl)serinate (formula 29). To a suspension of methyl serinate hydrochloride (6.43 mmol), 3,4-diacetoxycinnamic acid (12.86 mmol) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP-Cl) (13.1 mmol) in CH_2Cl_2 (120 mL) was added N-methylmorpholine (52.1 mmol), followed by a subsequent addition of 1-hydroxybenzotriazole hydrate (12.9 mmol). The resulting brownish solution was stirred at room temperature, (24 h) then partitioned between CH_2Cl_2(150 mL) and brine (2 x 50 mL). The organic extracts were dried (Na_2SO_4), taken to dryness and purified by silica gel flash column chromatography ($CHCl_3$ and $CHCl_3$:EtOAc) to afford formula 29 as a gum (1.56 g, 40% yield). ^{1}H NMR ($CDCl_3$) δ 7.67 (d, J = 15.6 Hz, IH), 7.27-7.48 (m, 3H), 6.55 (s, 1H), 6.48 (d, J = 15.6 Hz, 1H), 5.06 (m, 1H), 4.55 (m, 2H), 3.90 (s, 3H), 2.39 (s, 3H), 2.38 (s, 3H), 2.15 (s,

3H); HRMS m/z calcd. for $C_{19}H_{21}NO_9$ 408.129 (M+H): found 408.123. [Note: assignment of relative sites of acylation is not unambiguous.]

O-(3,4-dihydroxycinnamoyl)serine (formula 30). Treatment of formula 29 as previously described for the conversion of formulas 18 to 27 provided formula 30 as white solid (43 % yield); mp 202 °C (dec). ^1H NMR (DMSO-d $_6$) δ 12.6 (br s, 1H), 8.15 (br s, 1H), 8.10 (br s, 1H), 7.97 (d, J = 8.1 Hz, 1H), 7.41 (d, J = 15.7 Hz, 1H), 6.85-7.11 (m, 3H), 6.71 (d, J = 15.6 Hz, 1H), 5.11 (br s, 1H), 4.59 (m, 1H), 3.85 (q, J = 11.0 Hz, 2H); FABMS m/z 266 (M-H). Anal. ($C_{12}H_{13}NO_6$•0.5 H_20) C, H, N.

The analytical results of Example 1 are summarized in Table 2.

Table 2

No.	Formula	Theoretical	Found
8	$C_{30}H_{30}O_{12}$•H_20	C, 60.00; H, 5.37	C, 60.01; H, 5.14
9	$C_{32}H_{32}O_{12}$•H_20	C, 61.31; H, 5.47	C, 61.35; H, 5.37
10	$C_{28}H_{26}O_{12}$	C, 60.65; H, 4.73	C, 60.47; H, 4.76
11	$C_{29}H_{28}O_{12}$•H_2O	C, 59.38; H, 5.16	C, 59.48; H, 4.85
12	$C_{28}H_{21}NO_{11}$	C, 60.76; H: 4.92; N, 2.53	C, 60.61; H, 5.00; N, 2.41
13	$C_{22}H_{22}O_8$•H_2O	C, 61.11; H, 5.59	C, 61.46; H, 5.47
14	$C_{24}H_{24}O_8$•1$^1/_4$ H_2O	C, 62.26; H, 5.77	C, 62.02; H, 5.54
15	$C_{20}H_{18}O_8$•H_2O	C, 59.41; H, 4.99	C, 59.40; H, 4.67
16	$C_{21}H_{20}O_8$•H_2O	C, 59.38; H, 5.16	C, 59.48; H, 4.85
17	$C_{20}H_{19}NO_7$•0.5 H_2O	C, 60.91; H, 5.11; N, 3.55	C, 60.89; H, 5.10; N, 3.48
18	$C_{30}H_{28}O_{14}$	C, 58.82; H, 4.61	C, 58.52; H, 4.72
19	$C_{30}H_{29}NO_{13}$	C, 58.92; H, 4.78; N, 2.29	C, 58.65; H, 4.94; N, 2.20
20	$C_{30}H_{29}NO_{13}$•0.5 H_2O	C, 58.06; H, 4.87; N, 2.26	C, 57.99; H, 4.85; N, 2.33
21	$C_{30}H_{29}NO_{13}$ 0.5 H_2O	C, 58.06; H, 4.87; N. 2.26	C, 58.02; H, 4.69; N, 2.35
22	$C_{30}H_{30}N_2O_{12}$	C, 59.01; H, 4.95; N, 4.59	C, 59.02; H, 4.90; N, 4.54
23	$C_{26}H_{30}N_2O_8$	C, 62.64; H, 6.06; N, 5.62	C, 62.38; H, 5.99; N, 5.55
24	$C_{29}H_{26}O_{14}$•2.5 H_2O	C, 54.12; H, 4.86	C, 54.36; H, 4.68
25	$C_{29}H_{28}N_2O_{12}$	C, 59.27; H, 4.81; N, 5.35	C, 59.05; H, 4.95; N, 5.20
	•0.4 C_5H_5N		
26	$C_{25}H_{28}N_2O_8$	C, 61.97; H, 5.82; N, 5.78	C, 61.68; H, 5.86; N, 5.63
27	$C_{21}H_{18}O_{10}$•0.6 H_2O	C, 57.17; H, 4.39	C, 57.19; H, 4.72
28	$C_{21}H_{19}NO_9$•0.7 H_2O	C, 57.06; H, 4.65; N, 3.17	C, 57.01; H, 4.71; N, 3.08
29	$C_{19}H_{21}NO_9$•0.7 H_2O	C, 54.34; H, 5.38; N, 3.34	C, 54.10; H, 5.23; N, 3.79
30	$C_{12}H_{13}NO_6$•0.5 H_2O	C, 52.17; H, 5.11; N, 5.07	C, 52.19; H, 4.82; N, 4.94

Example 2

Assays of Chicoric Acid Analogues and Derivatives for Activity Against Integrase, for Cytoprotective Activity, and for Cytotoxicity

Preparation of oligonucleotide substrates. High-performance liquid chromatography purified oligonucleotides AE117 (5′ - ACTGCTAGAGATTTTCCACAC - 3′) (SEQ ID NO: 1) and AE118 (5′ - GTGTGGAAAATCTCTAGCAGT - 3′) (SEQ ID NO: 2) were purchased from Midland Certified Reagent Company (Midland, TX). The expression vector for the wild-type integrase was a generous gift of T. Jenkins and R. Craigie, Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Disorders, National Institutes of Health, Bethesda, MD. To analyze the extent of 3′-processing and strand transfer using 5' -end labeled substrates, AE118 was 5′ -end labeled with T_4 polynucleotide kinase (Gibco BRL, Gaithersburg, Md.) and [γ-^{32}P]ATP (Dupont-NEN). The kinase was heat inactivated, and AE117 was added to the same final concentration. The mixture was heated at 95°C, allowed to cool slowly to room temperature, and run on a G-25 Sephadex quick spin column (Boehringer Mannheim, Indianapolis, IN) to separate annealed double-stranded oligonucleotide from unincorporated label.

Integrase assays. The detailed *in vitro* assays used to study integrase inhibitors were recently described elsewhere. Mazumder et al., The Humana Press, Inc., Totowa, NJ (1997) (In press). In brief, integrase was preincubated at a final concentration of 200 nM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES (pH 7.5), 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (wt/vol), 7.5 mM $MnCl_2$, 0.1 mg of bovine serum albumin per ml, 10 nM 2-mercaptoethanol, 10% dimethyl sulfoxide (DMSO), and 25 mM MOPS (morpholinepropanesulfonic acid) (pH 7.2)) at 30°C for 30 minutes. The 5′ -end ^{32}P-labeled linear oligonucleotide substrate (20nM) was then added, and incubation was continued for an additional 1 h. Reactions were quenched by the addition of an equal volume (16 μL) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene

cyanol, 0.025% bromophenol blue). An aliquot (5 µL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M tris-borate (pH 8.3), 2 mM EDTA, 20% acrylamide, 8 M urea). Gels were dried, exposed in a PhosphorImager cassette, and analyzed with a Molecular Dynamics PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.). Percent inhibition was calculated using the following equation: % inhibition = 100 x [1 -(D-C)/(N-C)], where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processing product) or strand transfer products for DNA alone, DNA plus integrase, and integrase plus drug, respectively. The 50% inhibitory concentrations (IC_{50}) were determined by plotting the log of drug concentration versus percent inhibition and identifying the concentration which produced an inhibition of 50%.

XTT cytoprotection assay. Testing performed at the NCI AIDS Drug Screening Laboratory is based on a protocol described by Weislow et al. Weislow et al., "New soluble-formazan assay for HIV-1 cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS antiviral activity," J. Natl. Cancer Inst., 81, 577-586 (1989). In brief, all compounds were dissolved in DMSO and diluted 1:100 in cell culture medium. Exponentially growing T4 lymphocytes (CEM-SS cell line) were added at 5,000 cells per well. Frozen virus stock solutions were thawed immediately before use, suspended in complete medium to yield the desired multiplicity of infection, and added to the microtiter wells, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound served as a toxicity control, and infected and uninfected cells without the compound served as basic controls. Cultures were incubated at 37°C in a 5% CO_2 atmosphere for 6 days. The (2,3-bis-[methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazan production, and in addition were directly viewed microscopically for detection of cytotoxic effects and confirmation of protective activity.

Mechanistic and target-based assays. All positive control compounds for individual assays except AZTTP were obtained from the NCI chemical repository. The reference reagents for the individual assays are as follows; *attachment*: Farmatalia (NSC 65016) (Clanton et al., "Novel sulfonated and phosphonated analogs of distamycin which inhibit the replication of HIV," Anitviral Res., 27, 335-354 (1995)) and dextran sulfate (NSC 620255), *reverse transcriptase inhibition*: rAdT Template/primer-AZTTP (Sierra BioResearch, Tucson, AZ), rCdG Template/primer-UC38 (NSC 629243) (Bader et al., "Oxathiin carboxaminlide, a potent inhibitor of human immunodeficiency virus reproduction," Proc. Natl. Acad. Sci., 88, 6740-6744 (1991)), *protease inhibition*: KNI-272 (NSC 651714) (Kageyama et al., "In vitro anti-human immunodeficiency virus (HIV) activities of transition state mimetic HIV protease inhibitors containing allophenylnorstatine," Antimicrob. Agents Chemother., 37, 810-817 (1993)), *integrase inhibition*: ISIS 5320 (NSC 665353) (Buckheit et al., "Potent and specific inihibition of HIV envelope-mediated cell fusion and virus binding by G quartet-forming oligonucleotide (ISIS 5320)," AIDS Res. Hum. Retroviruses, 10, 1497-1506 (1994)) and DIBA-1 (NSC 654077) (Rice et al., "Inhibitors of HIV nucleocapsid protein zinc fingers as candidates for the treatment of AIDS," Science 270, 1194-1197 (1995)) a NCp7 Zn finger inhibitor.

Antiviral assay. Antiviral Assays performed independently of the AIDS Drug Screen. Antiviral activity was performed independently of the NCI AIDS Drug Screen with CEM-SS cells and HIV-1_{RF} (MOI = 0.1) [AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, Bethesda, MD] using the XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide) cytoprotection assay as previously described. Buckheit et al, supra; Rice et al, "The site of action of 3-nitrosobenzamide on the infectivity process of human immunodeficiency virus in lymphocytes," Proc. Natl. Acad. Sci. USA 90, 9721-9724 (1993). Effective antiviral concentrations providing 50% cytoprotection (EC_{50}), and cellular growth inhibitory concentrations causing 50% cytotoxicity (IC_{50}) were calculated. 3' -Azido-2' -3' -

dideoxythymidine (AZT) and dextran sulfate were utilized as reference compounds for antiviral activity.

Experiments designed to determine MOI sensitivity were carried out in the following manner. CEM-SS cells were pre-incubated for 15 to 30 min with various concentrations of test compounds. Serial dilutions of HIV-1_{RF} were added and virus adsorption carried out for 2 h. After 2 h media containing unabsorbed virus was removed and cultures were continued with the appropriate concentrations of compounds in media consisting of RPMI 1640, 10% FCs, 20 μg/mL gentamicin with 200 mM L-glutamate. At days 3, 6 and 9 of incubation sister plates were scored for syncytia formation and 100 μl of cell-free supernatant were collected for measurement of HIV-1 p24 by ELISA (AIDS Vaccine Program, National Cancer Institute-Frederick Cancer Research and Development Program, Frederick, MD). Cell viability was determined by XTT dye reduction. All calculations were corrected for residual p24 from the infection protocol. Results are shown in Table 3.

Table 3. Effects of multiplicity of infection on cytoprotective effects of 4 and 7.

Virus Dilution	**Day of Infection**		
	3	**6**	**9**
		EC_{50}(μM)[a]	
Compound 7			
1 : 50	>50	>50	>50
1 : 100	>50	>50	>50
1 : 200	>50	22.6	>50
1 : 400	n.d.[b]	19.6	29.4
Compound 4			
1 : 50	>100	>100	>100
1 : 100	>100	82.8	>100
1 : 200	>100	32	39.2
1 : 400	n.d.	9.8	27

[a]50% Effective concentration or concentration of compound required to protect 50% of cells against retroviral cytopathic effects.
[b]Not done.

Experiments designed to determine whether pretreatment of HIV-1 with selected chicoric acid analogues enhanced antiviral activity were carried out as follows. Briefly, 1 ml of HIV-1_{RF} stock was pre-incubated with 100 μM chicoric

acid analog, DIBA-1 (a virucidal nucleocapsid zinc finger inhibitor) or 10 μM AZT (reverse transcriptase inhibitor) for 90 minutes at 37°C. Following incubation, compound was removed by centrifugation (18,000 x g), 1h at 4°C, and the virus pellet re-suspended in RPMI 1640, 10% FCS, 20 μg/mL gentamicin-200 mM L-glutamate with or without supplemental compound. Virus was serially diluted onto CEM-SS cells (5000 cells/well), adsorbed for 2 h and cultures continued for 6 days with or without supplemental compound. Cell-free supernatants were collected for measurement of HIV-1 p24 by ELISA, and cell viability determined by the XTT dye reduction method. All calculations were corrected for residual p24 from the infection protocol. Results are shown in Table 4.

Table 4

Treatment	**Reciprocal of Virus Dilution % of Control**		
	100	333	1000
D-Chicoric acid (4)			
Add at infection	141	73	60
Pretreat only (100 μM)	108	59	168
Pretreat and add 10 μM	114	82	135
Pretreat and add 1 μM	56	92	50
Tetra-acetylated D-chicoric acid (7)			
Add at infection	141	81	60
Pretreat only (100 μM)	120	163	51
Pretreat and add 10 μM	149	39	38
Pretreat and add 1 μM	100	57	17
AZT			
Pretreat only (100 μM)	114	119	121
Add at infection	35	3	5
DIBA-1			
Pretreat only (100 μM)	31	4	6

HIV-1 cell and target-based assays. Latently infected tumor necrosis factor α(TNF-α) inducible U1 cells were used to determine the effects of the compounds on late phase virus replication as previously described. Turpin et al., "Inhibitors of human immunodeficiency virus type 1 zinc fingers prevent normal processing of Gag precursors and result in the release of noninfectious virus particles," J. Virol. 70, 6180-6189 (1996). Briefly, U1 cells (5 x 10^4 per 0.2 cm well) were simultaneously treated with 5 ng/mL TNFα and test compound. Cultures

were continued for 48 hours after which cell viability was determined by XTT dye reduction, and cell-free supernatants collected for determination of p24 by the ELISA method. The cell-based p24 attachment assay has been described in detail elsewhere. Rice et al., Science, surpa. Assays for activity against HIV-1 reverse transcriptase rAdT (template/primer) and rCdG (template/primer) using recombinant HIV-1 reverse transcriptase (a kind gift from S. Hughes ABL Basic Research NCI-FCRDS, Frederick, MD) have been previously described. Rice et al., "Inhibition of multiple phases of HIV-1 replication by a dithiane compound that attacks the conserved zinc fingers of retroviral nucleocapsid proteins," Antimicrob. Agents Chemother., 41, 419-426 (1997). The substrate cleavage of recombinant HIV-1 protease in the presence of test compounds was quantified using an HPLC-based methodology with the artificial substrate Ala-Ser-Glu-Asn-Try-Pro-Ile-Val-amide (Multiple Peptide Systems, San Diego, CA) as has been previously described. Rice et al., Science, supra; Rice et al., Antimicrob. Agents Chemother., surpa.

To address issues concerning antiviral activity, we extended our studies to include other viral strains and cell types. When L-chicoric acid, D-chicoric acid and their tetraacetyl esters were tested against HIV-1 (IIIB) replication, they were found to inhibit by 50% (EC_{50}) at concentrations ranging from 1.7 to 5.3 μM in MT-4 cells (Table 4). On the other hand, the compounds were found to be toxic to the MT-4 cells with 50% inhibitory concentrations (IC_{50}) ranging from 39.7 to 60 μMN, resulting in selectivity indices of approximately 10. HIV-2 (ROD) was shown to be 2- to 4-times less sensitive to the inhibitory effects of these compounds in comparison to HIV-1 (IIIB). The sulfated polysaccharide dextran sulfate and the reverse transcriptase inhibitor zidovudine (AZT) were tested in parallel (Table 5).

Table 5. Anti-HIV activity of select analogues

	EC_{50} (µm)[a]		IC_{50} (µM)[b]
Compound	HIV-1 (IIIa)	HIV-2 (ROD)	
L-Chicoric acid (D)	5.3±0.2	21.2	45.0±1.8
Tetra-acetyl L-chicoric acid (6)	4.1±0.3	10.1	45.0±1.1
D-Chicoric acid (4)	1.7±0.1	8.6±3.2	45.0±5.2
Tetra-acetyl D-chicoric acid (7)	3.9±1.2	5.1±1.9	60.0±5.2
Dextran sulfate[c]	0.4±0.2	0.2±0.1	>125
Zidovudine	0.005±0.002	0.005±0.002	100±25

[a]50% Effective concentration or concentration of compound required to protect 50% of cells against retroviral cytophatic effects.

[b]50% Inhibitory concentration or concentration of compound required to reduce MT-4 cell viability by 50%.

[c]Concentrations of dextran sulfate are expressed in µg/mL.

DISCUSSION OF RESULTS OF EXAMPLES 1 AND 2

Catechol-containing bis-aryl moieties are a significant structural component in many potent HIV integrase inhibitors. Frequently such compounds are characterized by two aryl units (at least one of which bears 1,2-bis-hydroxylation) separated by a central linker. Zhao et al., J. Med. Chem., supra. Although such analogues exhibit good inhibition against isolated HIV integrase, often corresponding protective effects in HIV-infected cells are not observed (Mazumder et al., Biochemistry, supra; Mazumder et al., J. Med. Chem., supra), perhaps at least partially as a result of limiting collateral cytotoxicity. The revelation that L-chicoric acid (formula D) exhibits both potent integrase inhibition in isolated enzyme preparations and provides protective effects in HIV-infected cells is therefore worthy of note, since its structure falls within the parameters of prior analogues which lack antiviral activity due to cellular toxicity. The study whose results are reported in the Examples herein was undertaken to examine features of L-chicoric acid responsible for this differential effect and to develop analogues which mimic their reported activities.

Chirality. One striking feature of L-chicoric acid is the presence of chiral centers in the central linker. Biological systems often discriminate between D- and L-isomers because the interactions of enantiomeric ligands with chiral host proteins are diastereomeric in nature. To our knowledge, previous reports have detailed the HIV integrase inhibitory potency of only L-chicoric acid (formula D). Robinson et al., Proc. Natl. Acad. Sci. USA, supra; McDougall et al., supra; Robinson et al., Mol. Pharmacol., supra; King et al., supra. In order to examine the inhibitory profile of D-(+)-chicoric acid (formula 4), we recently prepared this material from D-(-)-tartrate. (Zhao et al., Synthetic Commun., supra) (It should be noted that although L-chicoric acid exhibits a (-) sign of optical rotation, it bears the (2R,2R) absolute configuration of (+)-tartaric acid from which it is derived.) As shown in Figure 3, both L- and D-chicoric acids (formulas D and 4, respectively) are nearly identical in potency against integrase (IC_{50} values of approximately 1 μM). Both D- and L-chicoric acid were assessed by the AIDS Drug Screening Program of the NCI in a standardized XTT cytoprotection assay, which measures the ability of compounds to protect CEM-SS cells from the cytopathic effects of the HIV-1_{RF} virus strain. Weislow et al., supra. Neither compound significantly inhibited the replication of HIV-1 in this system. In both cases, cytoprotection either did not reach a 50% value (EC_{50}) or the antiviral effect was observed just prior to doses approaching the IC_{50} values (dose exhibiting 50% cell toxicity on uninfected cells). Since observed protection occurred in parallel with loss of cell viability, its significance is unknown.

Central linker carboxylic acid functionality. The chicoric acids are set apart from many other bis-aryl integrase inhibitors by having both a center of chirality and carboxylic acid functionality on their central linkers. To examine the importance of carboxyl groups, analogue formula 13 was prepared which lacks the carboxylic oxygens, yet maintains the chirality of L-chicoric acid (formula D). As shown in Figure 3, while integrase strand transfer inhibitory potency of formula 13 (IC_{50} = 0.5 μM) is moderately reduced relative to L-chicoric acid, significant potency is retained. As a further examination of formula 13, conformational constraint was introduced by incorporation of its central linker into a cyclohexane ring formula 14.

No apparent effect was observed on integrase strand transfer inhibitory potency. Finally, substituents were removed from the central linker formula 15, effectively eliminating chirality as well as any conformation constraints originally induced by such groups. This modification reinstated the high integrase inhibitory potency (IC_{50} = 0.5 μM) originally displayed by the parent chicoric acids formulas 3 and 4. Even though modifications of the bis-carboxylic acid linker resulted in significant changes in the ability of purified integrase enzyme to mediate 3′ processing and integration, all 3 compounds displayed significant cellular toxicity and lacked antiviral activity.

Central linker chain length and mode of caffeoyl-attachment. Two additional parameters were examined relative to analogue formula 15: chain length and mode of caffeoyl attachment. Modification of formula 15 by the addition of a methylene (formula 16) or replacing the caffeoyl ester linkage with an amide linkage (formula 17) resulted in significant losses in integration activity (27- and 23-fold decreases, respectively). Although for both formulas 16 and 17, good inhibitory potency was maintained against purified integrase, as with other members of this series, formulas 16 and 17 were toxic and failed to exhibit antiviral activity.

Tetra-acetate derivatives. The catechol moiety has proven critical for potent integrase inhibition over a wide range of inhibitor subclasses. This has been particularly true for those analogues which can be loosely grouped as "CAPE variants". Since the catechol structure could potentially contribute to unwanted collateral cytotoxicity (Stanwell et al., supra), it would be desirable to eliminate such functionality while maintaining integrase inhibitory potency. Recent efforts in this regard have largely been unsuccessful when applied to CAPE-type inhibitors. Burke et al., supra; Zhao et al., J. Med. Chem., supra. For example, derivatizing potent catechol-containing inhibitors as their methyl ethers has almost uniformly resulted in a loss of integrase inhibition. Burke et al., supra; Zhao et al., J. Med. Chem., supra. In contrast to these observations, L-chicoric acid has been reported to show antiviral activity in addition to integrase inhibition. Robinson et al., Proc. Natl. Acad. Sci. USA, supra; McDougall et al., supra; Robinson et al., Mol. Pharmacol., supra. Furthermore, recent induction of resistance to antiviral effects to L-chicoric acid in

HIV-1 infected cells by a single mutation of integrase, has increased support that L-chicoric acid exerts antiviral effects by inhibiting integrase. King et al, supra. We therefore examined the catechol functionality in this class of compounds as it relates to integrase inhibitory profile and antiviral activity. Tetra-acetates of L- and D-chicoric acid (formulas 6 and 7, respectively) (Zhao et al., Synthetic Commun., supra) were examined as chicoric acid analogues having their catechol functionality masked through ester formation. As shown in Figure 3, these non-catechol-containing derivatives displayed potent inhibition against purified integrase. As also shown in Figure 3, these non-catechol analogues also had reproducible low to moderate antiviral activity (formula 6::EC_{50} 16.8 μM, IC_{50} 48.8 μM, N=4; formula 7:EC_{50} 26.8 μM, IC_{50}23.2 μM, n=5) with slightly decreased cellular toxicity (formula D:IC_{50} 17.1μM).

Additional acetylated analogues of chicoric acid were prepared in analogy to the first series of unacetylated chicoric acid derivatives previously discussed. As reported above, while the catechol-containing analogues of the first series (formulas 13-17) displayed high integrase inhibitory potency in the absence of central linker carboxy functionality, this was not the case for acetylated chicoric acid analogues formulas 8 - 12. In this latter series, removal of this central linker functionality was accompanied by complete loss of inhibitory potency (Figure 3). For this class of compounds without central linker carboxyl groups, potent enzyme inhibition can only be obtained with catechol functionality. Stated another way, in the absence of catechol functionality (i.e., as tetra-acetates), central linker carboxyl functionality is required. Therefore simultaneous loss of both catechol and carboxylic functionality is not tolerated for integrase inhibition.

Monocarboxylic analogues. The parent chicoric acids are characterized by the presence of two carboxylic acid groups on their central linkers. As exemplified by the dicaffeoylquinic acids however, it has been shown that single carboxyls on a central linker between two caffeoyl esters can provide potent integrase inhibition. Robinson et al., Proc. Natl. Acad. Sci. USA, supra; McDougall et al., supra; Robinson et al., Mol. Pharmacol., supra; King et al., supra. Therefore,

it was of interest to examine what effect removal of one carboxylic group from the chicoric acid structures would yield.

Derivatives of glyceric acid, serine and β-aminoalanine were therefore prepared as monocarboxylic analogues of the parent chicoric acids. As shown in Figure 4, removal of one carboxyl group (fomula 27, $IC_{50} = 2$ μM) resulted in almost no loss of potency. Furthermore, replacement of one caffeoyl ester linkage by an amide linkage (formula 28, $IC_{50} = 2$ μM) provided equal potency. This latter transformation is significant in that formula 28 is now derived from an amino acid (serine). The tetra-acetates were also equally potent regardless of whether the caffeoyl groups were attached to the central linker by means of ester functionality (formula 24, $IC_{50}= 2$ μM) or amide groups (formula 25, $IC_{50}= 4$ μM).

Comparison of data in Figures 3 and 4 shows that tetra-acetates lacking central linker carboxyl groups (for example formulas 10 and 12) are inactive, while similar compounds containing a single central linker carboxyl group (formulas 24 and 25) exhibit full integrase inhibitory potency. It was of interest to investigate whether the anionic character of the carboxyl group was important for this inhibition. Therefore, methyl esters formulas 18 - 22 were prepared. While examining the role of a single carboxyl group, these methyl esters additionally served to investigate: (1) the importance of ester versus amide caffeoyl functionality in attaching the caffeoyl groups to the central linker (formulas 18, 19, and 22) and (2) the role of chirality at the α-carbon (formulas 20 and 21). As shown in Figure 11, methyl esters formulas 18 - 21 inhibited the integrase with nearly equal potency (IC_{50} values of approximately 2 - 4 μM), with formula 2 being only slightly less potent ($IC_{50}= 10$ μM). These values are essentially unchanged from the parent free carboxylic acids (formulas 27 and 28).

Importance of acetate functionality. Catechol functionality has been critical for integrase activity throughout many classes of compounds. For caffeic acid-containing inhibitors, masking the catechol groups has usually resulted in a loss of inhibitory potency. In light of this, the ability of the current series of

tetra-acetylated chicoric acid analogues to maintain inhibitory potency was unusual. It therefore seemed possible that the inhibitory potency of the tetra-acetates in the current series may be due to conversion of the acetates to the free catechols under the conditions of the enzyme assay. To examine this possibility, tetra-acetate formula 25 was incubated under the same conditions as those employed for the integrase assay. Using HPLC analysis, it was observed that formula 25 was stable during the time course of the assay, indicating that the chemical hydrolysis did not occur, and that enzyme inhibition was due to the tetra-acetate itself and not hydrolysis products.

It was also noted that in previous studies, masking of catechol functionality was often achieved by converting the catechol hydroxyls to their methyl ethers. In most cases the resultant ethers showed significantly reduced integrase inhibitory potency relative to the parent catechols. Burke et al., supra; Zhao et al., J. Med. Chem., supra. In the present series, the question therefore arose whether tetra-methyl ethers would maintain high inhibitory potency similar to the tetra-acetate esters. To examine this question, tetra-methyl ethers formulas 23 and 26 were prepared. As shown in Figure 4, in contrast to the high inhibitory potency of the parent tetra-acetates formulas 22 and 25 respectively, the tetra-methyl ethers were inactive. These results indicate that functionality within the acetate esters themselves not found in the methyl ethers, is critical for high inhibitory potency.

Importance of two caffeoyl groups. Finally, it was of interest to determine whether two caffeoyl groups were required for high inhibitory potency. To examine this question, mono-caffeoyl analogues formulas 30 and 29 were prepared as free catechol and as acetylated variants, respectively. The inactivity of both analogues (Figure 4) indicates that two caffeoyl moieties are important for potency within the series. In summary however, even though definite structural correlates to integrase inhibitory activity could be identified, no analogues with demonstrable antiviral efficacy were obtained.

Effects of divalent metal ions. To examine the role of divalent metals in the inhibition of HIV-1 integrase by the chicoric acids we performed the assays in the presence of Mn^{+2} and Mg^{-2}. Six representative formulas, D, 4, 6, 15, 25, and 27 containing none, one or two carboxyl groups were examined. Reaction conditions were optimized for each divalent metal as described. The Mn^{+2} assays were performed in the presence of 7.5 mM manganese chloride. The Mg^{+2} assays were performed in the presence of 5% polyethylene glycol and 7.5 mM magnesium chloride. A representative gel from these experiments is shown in Figures 5A and 5B; the data is summarized in the graphs of Figures 5C and 5D.

The effect of metals on the inhibition of integrase by these compounds is striking. First, when inhibition assays were run in the presence of Mn^{+2}, all compounds inhibited the enzyme within the same range of concentration (IC_{50} range 1 - 10 μM). Therefore, effects related to the central linker (number of carboxyl groups) and free or acetylated catechols were minimal in the presence of Mn^{+2}. However, when Mg^{+2} was substituted for Mn^{+2}, formulas 15 and 25 were inactive while formula 27 exhibited 10-fold reduction in potency, suggesting that for maximal potency in the Mg^{+2} assays at least two-carboxyl groups are required. Taken together, our data indicate that the chelation of divalent metals on the active site of integrase may be responsible for the differential potency of these compounds in the presence of different metals. It is also plausible that the integrase enzyme could adopt a different folding pattern in the presence of various metals. Studies are underway to elucidate the role of metals in the inhibition of integrase function.

Interpretation of cell-based data. A large number of the catechol-containing compounds generated in this study, including D- and L-chicoric acid, were found to be toxic on CEM-SS cells and lacked significant antiviral activity in the XTT cytoprotection assay. Previous reports of L-chicoric acid as a potent antiviral agent in syncytia-based assays (Robinson et al., Proc. Natl. Acad. Sci. USA, supra; McDougall et al., supra; Robinson et al., Mol. Pharmacol., supra) prompted us to investigate these results more thoroughly.

Formulas D, 4, 6 and 7 were independently assessed on 3 separate occasions for antiviral activity in the XTT cytoprotection assay using CEM-SS cells, viral stocks and protocols utilized by the formal NCI screen. Weislow et al., supra. The data shows that all 4 compounds failed to achieve 50% protection. The assays of were performed as previously described. Rice et al., Proc. Natl. Acad. Sci. USA, supra; Rice et al., Antimicrob. Agents Chemother., supra. Formulas 6 and 7 did elicit slight cytoprotection, but dose limiting toxicities prevented reaching an EC_{50} value for either compound. Even though consistent antiviral activity could not be identified, these compounds were further examined in target and cell-based assays to determine their mechanism of action. The four compounds were independently found to inhibit integrase function, but failed to significantly inhibit virus attachment, reverse transcriptase activity or protease activity. In addition the compounds failed to inhibit viral replication in Tumor Necrosis Factor-α (TNF-α)-induced latently infected U1 cells, indicating that any possible antiviral activity was not associated with a post-integration target.

Formulas 4 and 7 were reassessed for antiviral activity under more rigorous conditions to determine if our failure to demonstrate antiviral activity as previously reported (Robinson et al., Proc. Natl. Acad. Sci. USA, supra) was an artifact of our detection system. The role of multiplicity of infection (MOI) in the efficacy of these compounds was determined first (Table 3). CEM-SS cells were pretreated for 30 minutes with various doses of formulas 4 and 7 after which the cells were infected with serial dilutions of HIV-1 RF. Cultures were continued in media supplemented with compound for 3, 6 and 9 days, after which supernatants were collected and p24 antigen content measured by ELISA. The resulting values were corrected for input p24 antigen (infecting stock) and normalized to percent control (Table 3). Inhibition of HIV-2 replication by formulas 4 and 7 was highly dependent on the MOI, with moderate antiviral activity associated with only the high-test virus dilutions at 6 days of infection (Table 3). Additionally, extension to 9 days of culture revealed that the antiviral effects measured at 6 days were transient.

A requirement for pre-incubation of the virus and compound for optimal antiviral activity has recently been identified. McDougall et al., supra. Therefore, a pooled stock HIV-1_{RF} was pretreated for 90 minutes at 37°C with 100 μM of either formulas 4 or 7, after which excess compound was removed by centrifugation (18,000 x g, 4°C, 1 h), and cultures continued with or without re-addition of 10 or 1 μM fresh compound (Table 4). In order to ensure optimal ratios of virus to target cells, the cells were treated with multiple dilutions of the treated virus. Supernatants were collected at 7 days of culture and virus replication assessed by p24 ELISA. Formula 4 at 100 μM had no appreciable effect on viral replication, while at very low MOIs (low input titers of virus), while at very low MOIs (low input titers of virus) formula 7 at 100 μM reduced, but did not completely inhibit virus replication. Pretreatment with 50 μM failed to elicit inhibitory activity with either analogue (data not shown). Pretreatment of virus with AZT failed to inhibit virus replication (cell-mediated metabolism is required to convert AZT into its active form, AZT-TP), while simultaneous addition with virus and cells and maintenance in cell culture resulted in >95% inhibition of virus replication at 1:333 and 1:1000 dilutions of virus. DIBA-1 (Rice et al, Science, supra), a directly viricidal NCp7 Zn finger reactive compound, also resulted in >95% inhibition of virus replication at 1:333 and 1:1000. Thus, although target-based assays have identified chicoric acid derivatives as potent inhibitors of purified HIV-1 integrase, demonstration of their weak antiviral activity is highly dependent upon the MOI and order of drug addition.

Conclusions

L-chicoric acid (formula D) and the quinic acids have emerged as interesting classed of caffeoyl-containing catechols exhibiting potent HIV integrase inhibition. These analogues fall within the general category of inhibitors categorized by two aryl groups separated via a central linker, with at least one of the aryl groups being a catechol. While there are a large number of inhibitors within this general class, the multiple reports of anti-HIV activity of L-chicoric acid and quinic acids in cell-based assays has placed them apart from other catechols, which frequently

exhibit limiting cytotoxicity. The present study was undertaken to examine features of L-chicoric acid that contribute both to its integrase enzyme inhibition and cell-based activity. At the outset of this work, structural features of L-chicoric acid potentially important for biological efficacy included: (1) *Chirality*; only the L-enantiomer had been examined for inhibitory potency, (2) *Carboxylic acids*; the presence of two carboxylic acids on a central linker, (3) Ester linkages; attachment of two caffeoyl groups by ester bonds, (4) *Catechols*; the presence of two catechol rings.

Through a progressive series of analogues, we have shown against purified integrase that enantiomeric D-chicoric acid (formula 4) retains integrase inhibitory potency equal to its L-counterpart and further that removal of either one (formula 27) or both carboxylic functionalities (formula 15) results in essentially no loss of integrase inhibitory potency. Additionally, while two caffeoyl moieties are required, (mono-caffeoyl analogues formula 29 and 30 are inactive), attachment of caffeoyl groups to the central linking structure can be achieved via amide or mixed amide/ester linkages. More remarkable is the finding that blockade of the catechol functionality through conversion to tetra-acetate esters results in almost no loss of potency, contingent on the presence of at least one carboxyl group on the central linker. This single carboxyl group can be either the free acid or a methyl ester. Taken as a whole, the work has resulted in the identification of new integrase inhibitors typified by formulas 19 - 22, 25 and 28, which may be regarded as bis-caffeoyl derivatives of glycidic acid and amino acids such as serine and β-aminoalanine.

While such analysis of chicoric acid-type compounds have identified specific structure-function relationships between these molecules and their ability to inhibit purified integrase, examination of their antiviral activity revealed only very modest inhibitory activity under highly specific conditions. The XTT cytoprotection assay is a well established standardized procedure for determining antiviral activity of unknown compounds. The failure of an unknown to mediate antiviral activity may be due to a number of factors. For the purpose of discussion these factors can

be divided into intrinsic (assay determined) and extrinsic (assay independent) assay factors. In the XTT cytoprotection assay, the most common extrinsic assay factor results from interaction of the XTT dye with the compound under investigation, resulting in quenching or abnormally high optical densities. Chicoric acid derivative concentrations above 200 μM were found to interfere with the XTT cytoprotection assay by depressing the observed optical absorbances. Even though interference occurred at analogue concentrations of approximately 10 times the observed experimental IC50 (concentration result in 50% cell death) these results led to analysis of subsequent assays by ELISA determination of p24 antigen expression. Measurement of p24 antigen was slightly more sensitive than the XTT assay, but resulted in the same general outcome for the antiviral assays.

Compounds may fail in the XTT cytoprotection assay due to intrinsic assay factors such as the multiplicity of infection (MOI, ratio of virus particles to target cell) and the timing of compound addition in relation to the infection event. These two parameters were investigated in detail (Tables 3−4). Antiviral activity was found to be MOI dependent, requiring preincubation of the virus and chicoric acid analogue followed by subsequent culture in compound−containing media to elicit maximal antiviral activity. Even with optimal MOI's and continuous exposure to the chicoric acid analogues, antiviral activity was far less potent than that mediated by either AZT or DIBA−1. Furthermore under optimized conditions inhibition was transient. Thus, the chicoric acid mediated inhibition of HIV−1 replication is far less robust and requires extensive in vitro manipulation to elicit antiviral activity that is inferior to known and well characterized antivirals targeting reverse transcriptase and the NCp7 Zn finger. However, better antiviral activities were observed against HIV−1(IIIB) and HIV−2(ROD) in MT−4 cells (Table 5), and this fact will be the object of future studies directed at elucidating mechanism(s) by which these compounds exert their antiviral effects.

Example 3

Rapid Syntheses of (2R,3R)–(–)– and (2S,3S)–(+)–Chicoric Acids

For the synthesis of (2R,3R)–(–)–chicoric acid formula 32, a solution of commercially available (+)–di–t–butyl L–tartrate formula 34 in anhydrous pyridine (3 ml to 1 mmol of tartrate) was treated with the solution of 2 equivalent of freshly prepared 3,4–diacetyl caffeoyl acid chloride formula 35 in toluene (5 mL to 1 mmol of tartrate) at room temperature overnight (the compounds used in this synthetic route are depicted in Table 1). The 3,4–diacetyl caffeoyl acid chloride has been previously prepared (E. Haslam et al., J. Chem. Soc. 1964: 2137). It is easily synthesized on large scale by treatment of 3,4–dihydroxycinnamic acid with acetic anhydride (10 equivalents) in pyridine (v/v = 5:1) in the dark overnight at room temperature. Upon removal of solvent, residue is crystallized from EtOAc:hexane to provide diacetyl caffeic acid as a white solid. Reflux (3 h) with thionyl chloride (5 equivalents) in benzene (v/v = 8:1) provides 3,4–diacetyl caffeoyl acid chloride upon removal of solvent.

After removal of pyridine, the residue was dissolved in toluene and evaporated under reduced pressure. Two subsequent additions and evaporations of toluene eliminated residual pyridine. The resulting material was passed through silica gel (EtOAc:hexane 1:1) to provide formula 36 as a white solid (97% yield). Mp 153–155°C; $[\alpha]^{25}_{D}$ 146° ($CHCl_3$, c 0.51); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.73 (d, J = 16.0 Hz, 2H), 744–7.36 (m, 4H), 7.24 (d, J =8.3 Hz, 2H), 6.50 (d, J =16.0 Hz, 2H), 5.79 (s, 2H), 2.30 (s, 6H), 2.28 (s, 6H), 1.41 (s, 18H); FABMS m/z 756 $(MH)^+$. Anal. calcd. for $C_{28}H_{42}O_{16}$: C, 60.47, H, 5.61. Found, C, 60.38, H, 5.65.

Formula 36 was treated with TFA (20 equivalents) in dichloromethane (v/v = 1:3) at room temperature overnight, then either crystallized (acetone:hexane) to provide formula 37 as a white solid (Mp 186–188°C; $[\alpha]^{25}_{D}$ –159° (MeOH, c 0.16); ^{1}H NMR (250 MHz, DMSO–d_6) δ 7.81 (d, J = 1.8 Hz, 2H), 7.76–7.70 (m, 4H), 7.34 (d, J = 8.4H, 2H), 6.78 (d, J = 16.2 Hz, 2H), 5.74 (s, 2H), 2.28 (s, 12H); FABMS m/z 644 $(MH)^+$. Anal. calcd. for $C_{30}H_{26}O_{16}$: C, 56.08, H, 4.08. Found, C, 55.82, H, 4.17) (96%) or directly taken to dryness and hydrolyzed with 3 N HCl (10 mL to 1 mmol of formula 36) in acetone (v/v: 1:3) under reflux (3 h). After cooling to room temperature, the solution was diluted with EtOAc (5 volumes), washed with brine and dried over anhydrous sodium sulfate. Solvent was

removed and residue crystallized from H_2O to provide (2R,3R)−(−)chicoric acid formula 32 (90% yield). Mp 204−206°C; $[\alpha]^{25}_D$ −333° (MeOH, c 0.10; lit. (M.L. Scarpati & G. Oriente, Tetrahedron 4: 43−48 (1958)) −384.2°,(MeOH, c 1.075); ^{1}H NMR (250 MHz, DMSO−d_6) δ 9.69 (s, 2H), 9.16 (s, 2H), 7.55 (d, J = 15.8 Hz, 2H), 7.07−7.07 (m, 4H), 6.77 (d, J = 8.6 Hz, 2H), 6.36 (d, J = 15.9 Hz, 2H), 5.67 (s, 2H); FABMS m/z 475 $(MH)^+$. Anal. calcd. for $C_{22}H_{18}O_{12}$•7/4H_2O:C, 52.23, H, 42.8. Found, C, 52.07, H, 4.33. Enantiomeric natural (2S,3S)-chicoric acid formula 31 was obtained in an identical fashion starting from commercially available (-)-di-t-butyl D-tartrate ($[\alpha]^{25}_D$ +340° (MeOH, c 0.14); other physical data identical to that displayed by the enantiomer).

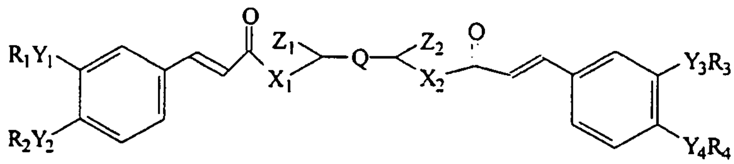
This synthesis is an improvement on the originally reported synthesis which was achieved in low yield using the highly unstable acid chloride formula 33 (M.L. Scarpati et al., supra; W.E. Robinson, Jr., et al., Proc. Natl. Acad. Sci. USA 93: 5326−6331 (1996)). This synthesis avoids the preparation or isolation of the acid chloride or the tartaric acid bis−diphenyl ester, which was found to be difficult. This synthesis therefore employs alternate protection strategies. In the case of tartaric acid, the bis−t−butyl ester was chosen, because the ester functionality can be removed under mild acidic conditions, and the material is commercially available. For dihydroxycinnamic acid, the acid chloride, bearing acetyl phenolic protection, was utilized because it was easily prepared and isolated. Acylation of formulas 34 with 35 in pyridine provided fully protected intermediate as a crystalline solid in nearly quantitative yield. Removal of protecting groups was then achieved in a two−step process initiated by TFA−mediated hydrolysis of t−butyl esters to yield free diacid formula 37. Treatment with 3 N HCl in acetone then provides the desired (−)−chicoric acid formula 32 as a crystalline solid in greater than 80% overall yield from starting commercially available tartrate formula **34**.

The method of this example is adaptable to the preparation of a wide range of chicoric acid analogues and derivatives, including those recited herein.

While the invention has been described in the above specification, examples and data, it is to be understood that the invention is not limited to the disclosed embodiments, but is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims. All references cited herein are hereby incorporated by reference in their entirety.

WE CLAIM:

1. A compound of formula (I)



where:

Q is a valence bond or CH_2;

X_1 is O, NH, or CH_2;

X_2 is O, NH, or CH_2;

Y_1, Y_2, Y_3, and Y_4 are each a valence bond, O, or NH;

where Y_1, Y_2, Y_3, or Y_4 is a valence bond, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is a carboxy–containing moiety selected from the group consisting of carboxymethyl, carboxyethyl, carboxypropyl, carboxy small alkyl and carboxy aryl;

where Y_1, Y_2, Y_3, or Y_4 is O, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each H, acetyl, propionyl, butyryl, or isobutyryl, or is a moiety forming a lower alkyl carbamate or an aryl carbamate;

where Y_1, Y_2, Y_3, or Y_4 is NH, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each acetyl, propionyl, butyryl, isobutyryl, small alkyl or aryl;

Z_1 and Z_2 are each H, lower alkyl, –CHO, $-CO_2H$, or $-CO_2W$, where W is lower alkyl or aryl, or, alternatively, where Q is a valence bond, Z_1 and Z_2, together with the adjacent carbon atoms and Q, form a ring structure, the carbon skeleton of the ring structure being selected from the group consisting of cyclohexane, cyclohexene, cyclopentane, cycloheptane, cycloheptene, and benzene;

with the proviso that where each of Y_1, Y_2, Y_3, or Y_4 is O and all of R_1, R_2, R_3, and R_4 are other than H, at least one of Z_1 or Z_2 is $-CO_2H$ or $-CO_2W$;

and with the proviso that where X_1 and X_2 are both O, Q is a valence bond, and Z_1 and Z_2 are both $-CO_2H$, either at least one of R_1, R_2, R_3, and R_4 is other than H or at least one of Y_1, Y_2, Y_3, and Y_4 is other than O.

2. The compound of claim 1 wherein the compound is L–chicoric acid tetraacetate.

3. The compound of claim 1 wherein the compound is D–chicoric acid tetraacetate.

4. The compound of claim 1 wherein the compound is (+)–(2S,3S)–O,O–bis–(3,4–dihydroxycinnamoyl)–2,3–butanediol.

5. The compound of claim 1 wherein the compound is (+)–(1S,2S)–O,O–bis–(3,4–dihydroxycinnamoyl)–1,2–cyclohexanediol.

6. The compound of claim 1 wherein the compound is O,O–bis–(3,4–dihydroxycinnamoyl)–1,2–ethanediol.

7. The compound of claim 1 wherein the compound is O,O–bis–(3,4–dihydroxycinnamoyl)–2,3–dihydroxypropanoic acid.

8. The compound of claim 1 wherein the compound is N,O–bis–(3,4.dihydroxycinnamoyl)serine.

9. The compound of claim 1 wherein the compound is O,O–bis–(3,4–diacetoxycinnamoyl)–2,3–dihydroxypropanoic acid.

10. The compound of claim 1 wherein the compound is N,N–bis–(3,4–diacetoxycinnamoyl)–2,3–diaminopropanoic acid.

11. The compound of claim 1 wherein the compound is methyl O,O–bis–(3,4–diacetoxycinnamoyl)–2,3–dihydroxypropanoate.

12. The compound of claim 1 wherein the compound is methyl N,O–bis–(3,4–diacetoxycinnamoyl)serinate.

13. The compound of claim 1 wherein the compound is methyl N,O–bis–(3,4–diacetoxycinnamoyl)–L–serinate.

14. The compound of claim 1 wherein the compound is methyl N,O–bis–(3,4–diacetoxycinnamoyl)–D–serinate.

15. The compound of claim 1 wherein the compound is methyl N,N–bis–(3,4–diacetoxycinnamoyl)–2,3–diaminopropanoate.

16. A method for inhibiting HIV–1 integrase comprising contacting HIV–1 integrase with a quantity of a compound sufficient to produce a detectable inhibition of HIV–1 integrase, the compound being a compound of formula (I) where:

Q is a valence bond or CH_2;

X_1 is O, NH, or CH_2;

X_2 is O, NH, or CH_2;

Y_1, Y_2, Y_3, and Y_4 are each a valence bond, O, or NH;

where Y_1, Y_2, Y_3, or Y_4 is a valence bond, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is a carboxy–containing moiety selected from the group consisting of carboxymethyl, carboxyethyl, and carboxypropyl;

where Y_1, Y_2, Y_3, or Y_4 is O, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each H, acetyl, propionyl, butyryl, or isobutyryl, or is a moiety forming a lower alkyl carbamate or an aryl carbamate;

where Y_1, Y_2, Y_3, or Y_4 is NH, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each acetyl, propionyl, butyryl, or isobutyryl;

Z_1 and Z_2 are each H, lower alkyl, $-CHO$, $-CO_2H$, or $-CO_2W$, where W is lower alkyl, or, alternatively, where Q is a valence bond, Z_1 and Z_2, together with the adjacent carbon atoms and Q, form a ring structure, the carbon skeleton of the ring structure being selected from the group consisting of cyclohexane, cyclohexene, cyclopentane, cycloheptane, cycloheptene, and benzene;

with the proviso that where each of Y_1, Y_2, Y_3, or Y_4 is O and all of R_1, R_2, R_3, and R_4 are other than H, at least one of Z_1 or Z_2 is $-CO_2H$ or $-CO_2W$;

and with the proviso that either: (1) where X_1 and X_2 are both O, Q is a valence bond, and Z_1 and Z_2 are both $-CO_2H$, either at least one of R_1, R_2, R_3, and R_4 is other than H or at least one of Y_1, Y_2, Y_3, and Y_4 is other than O, or, (2) that where X_1 and X_2 are both O, Q is a valence bond, Z_1 and Z_2 are both $-CO_2H$, all of R_1, R_2, R_3, and R_4 are H, and all of Y_1, Y_2, Y_3, and Y_4 are O, the compound is the D–(+) enantiomer.

17. A method for administering to a patient infected with HIV−1 comprising administering to the patient a compound in a quantity sufficient to inhibit the replication of HIV−1 by detectably inhibiting the activity of HIV−1 integrase in the patient, the compound being a compound of formula (I) where:
Q is a valence bond or CH_2;
X_1 is O, NH, or CH_2;
X_2 is O, NH, or CH_2;
Y_1, Y_2, Y_3, and Y_4 are each a valence bond, O, or NH;
where Y_1, Y_2, Y_3, or Y_4 is a valence bond, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is a carboxy−containing moiety selected from the group consisting of carboxymethyl, carboxyethyl, and carboxypropyl;
where Y_1, Y_2, Y_3, or Y_4 is O, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each H, acetyl, propionyl, butyryl, or isobutyryl, or is a moiety forming a lower alkyl carbamate or an aryl carbamate;
where Y_1, Y_2, Y_3, or Y_4 is NH, the element R_1, R_2, R_3, or R_4 bonded to Y_1, Y_2, Y_3, or Y_4 is each acetyl, propionyl, butyryl, or isobutyryl;
Z_1 and Z_2 are each H, lower alkyl, $-CHO$, $-CO_2H$, or $-CO_2W$, where W is lower alkyl, or, alternatively, where Q is a valence bond, Z_1 and Z_2, together with the adjacent carbon atoms and Q, form a ring structure, the carbon skeleton of the ring structure being selected from the group consisting of cyclohexane, cyclohexene, cyclopentane, cycloheptane, cycloheptene, and benzene;
with the proviso that where each of Y_1, Y_2, Y_3, or Y_4 is O and all of R_1, R_2, R_3, and R_4 are other than H, at least one of Z_1 or Z_2 is $-CO_2H$ or $-CO_2W$;
and with the proviso that either: (1) where X_1 and X_2 are both O, Q is a valence bond, and Z_1 and Z_2 are both $-CO_2H$, either at least one of R_1, R_2, R_3, and R_4 is other than H or at least one of Y_1, Y_2, Y_3, and Y_4 is other than O, or, (2) that where X_1 and X_2 are both O, Q is a valence bond, Z_1 and Z_2 are both $-CO_2H$, all of R_1, R_2, R_3, and R_4 are H, and all of Y_1, Y_2, Y_3, and Y_4 are O, the compound is the D−(+) enantiomer.

18. A composition comprising:

(a) the compound of claim 1 in a quantity sufficient to inhibit at least one reaction of HIV−1 integrase selected from the group consisting of 3′−processing and strand transfer; and

(b) an acceptable carrier.

19. A process for synthesizing chicoric acid or a chicoric acid analogue or derivative having two free carboxyl groups in its central linker comprising the steps of:

(a) reacting di-t-butyl tartrate with the acid chloride of a protected derivative of dihydroxycinnamic acid having its phenolic hydroxyl groups acylated;

(b) removing the di-t-butyl groups; and

(c) removing the acyl groups protecting the phenolic hydroxyls to produce the chicoric acid or chicoric acid analogue or derivative having two free carboxyl groups in its central linker.

20. A process for synthesizing a chicoric acid analogue or derivative having no free carboxyl groups in its central linker comprising the steps of:

(a) reacting the acid chloride of a protected derivative of dihydroxycinnamic acid having its phenolic hydroxyl groups acylated with a diol or ethanolamine; and

(b) removing the acyl groups protecting the phenolic hydroxyls to produce the chicoric acid analogue or derivative having no free carboxyl groups in its central linker.

21. A process for synthesizing a chicoric acid analogue or derivative having one free carboxyl group in its central linker comprising the steps of:

(a) reacting the acid chloride of a protected derivative of dihydroxycinnamic acid having its phenolic hydroxyl groups acylated with a dihydroxy compound that is further substituted with an alkyl ester group;

(b) hydrolyzing the alkyl ester group to a carboxylic acid; and

(c) removing the acyl groups protecting the phenolic hydroxyls to produce the chicoric acid analogue or derivative having one free carboxyl group in its central linker.

FIG. 1

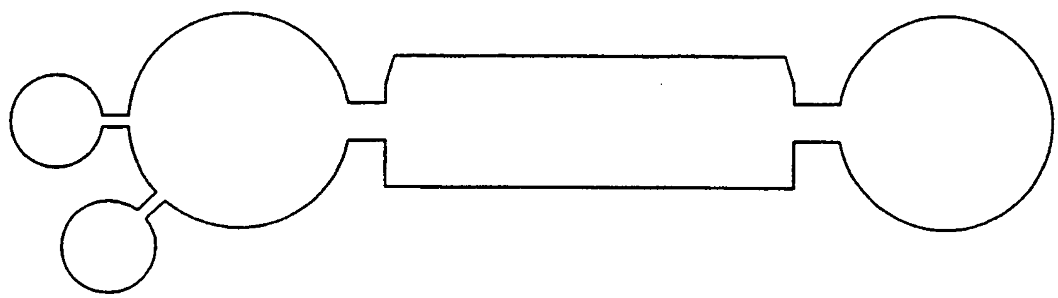
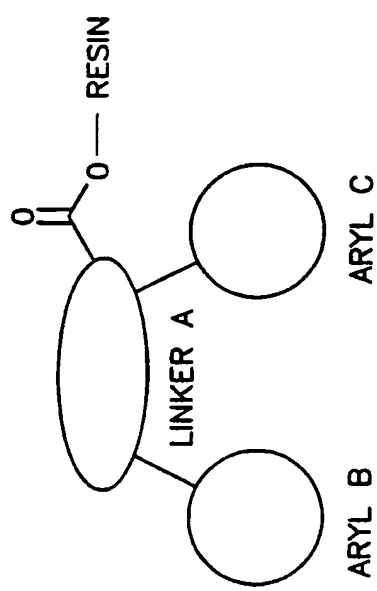
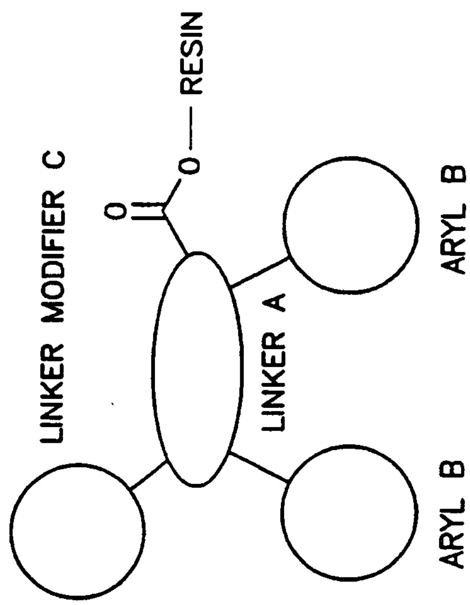


FIG. 2A



3 COMPONENTS USING DIFFERENT ARYL GROUPS

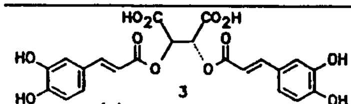
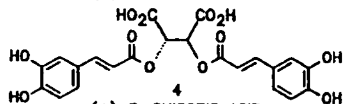
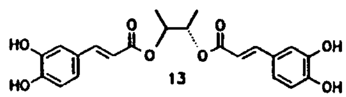
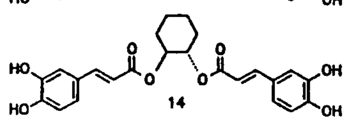
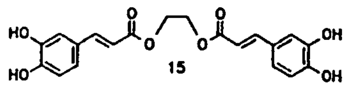
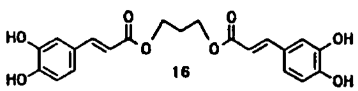
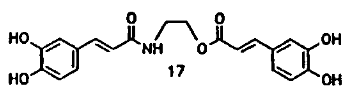
FIG. 2B

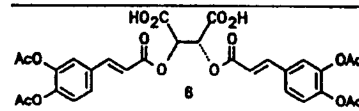
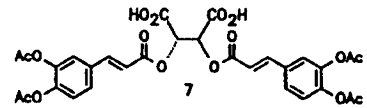
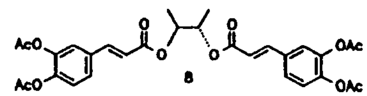
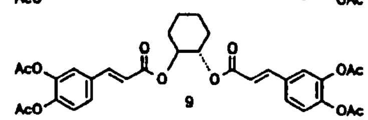
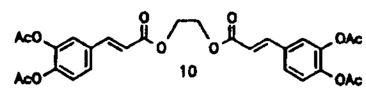
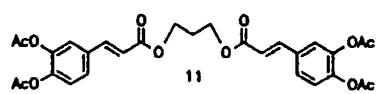
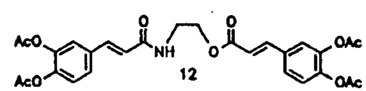


3 COMPONENTS USING IDENTICAL ARYL GROUPS,
BUT WITH A "LINER MODIFIER"

FIG. 3

RESULTS OF RADIONUCLEOTIDE–BASED INTEGRASE ASSAYS AND CYTOPROTECTION IN THE NCI AIDS SCREEN.

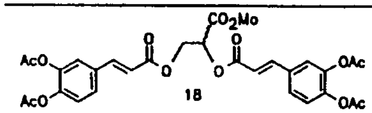
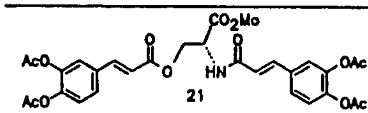
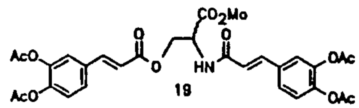
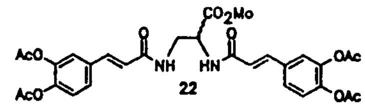
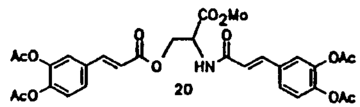
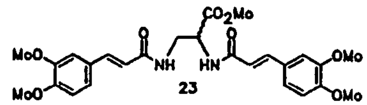
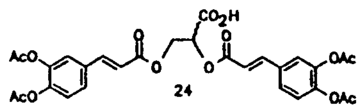
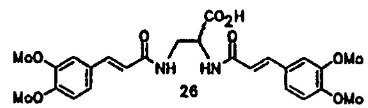
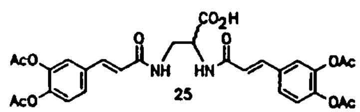
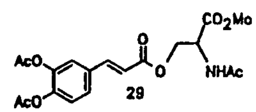
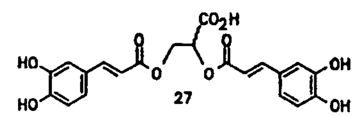
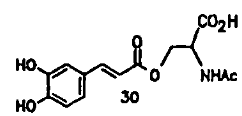
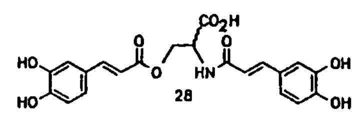
STRUCTURE	INTEGRASE ASSAY[a] (IC_{50} µM) 3'–PROCESSING	INTEGRATION	CELL DATA[b] (µM) IC_{50}	EC_{50}	TI_{50}
 3 (–)–L–CHICORIC ACID	1.1(2.3)	0.8(1.0)	20.1 14.2	>20.1 >14.2	– –
 4 (+)–D–CHICORIC ACID	1.1	0.5	16.0 35.5	16.7 13.6	1.0 2.6
 13	38.4(>10)	7.8(7.4)	3.30 3.64	>3.30 >3.84	– –
 14	24.8(>10)	7.8(6.1)	5.67	>5.67	–
 15	0.4(0.9)	0.4(0.3)	9.69 10.0	>9.69 >10.0	– –
 16	27.5(16)	7.5(6.4)	13.5 13.9	>13.5 >13.9	– –
 17	27.5(>10)	6.5(6.1)	9.22 7.71	>9.22 >9.7	– –

STRUCTURE	INTEGRASE ASSAY[a] (IC_{50} µM) 3'–PROCESSING	INTEGRATION	CELL DATA[b] (µM) IC_{50}	EC_{50}	TI_{50}
 6	9.8(10)	6.2(7)	51.4 54.7 48.8 44.9	12.0 16.2 20.3 18.9	4.3 3.4 2.4 2.4
 7	33.3(10)	4.6(5.2)	30.4 16.7 13.6 30.5 25.0	16.8 16.0 36.5 18.6 46.3	0.5 1.0 2.6 5.1 1.8
 8	>333	>333	5.45 5.71	>5.45 >5.71	– –
 9	>333	>333	5.74 5.76	>5.74 >5.76	– –
 10	>100	>100	3.8 5.49 8.3 9.3	>3.8 >5.49 >8.3 >9.3	– – – –
 11	>333	>333	–	–	–
 12	>333	100	14.8 15.6	>14.8 >15.6	– –

[a] DETERMINED BY RADIOLABLED OLIGONUCLEOTIDE–BASED ASSAY AS DESCRIBED IN THE EXPERIMENTAL SECTION. VALUES IN PARENTHESIS, OR DUPLICATE VALUES ARE THE RESULTS OF MULTIPLE RUNS
[b] DETERMINED THROUGH THE NCI AIDS SCREEN USING AN XTT CYTOPROTECTION ASSAY. REPLICATE VALUES ARE THE RESULTS OF MULTIPLE RUNS.

FIG. 4

RESULTS OF RADIONUCLEOTIDE-BASED INTEGRASE ASSAYS.

STRUCTURE	INTEGRASE ASSAY[a] (IC_{50} µM) 3'-PROCESSING	INTEGRATION	STRUCTURE	INTEGRASE ASSAY[a] (IC_{50} µM) 3'-PROCESSING	INTEGRATION
 18	10.1(10.0)	10.1(8.0)	 21	2.8(3.0)	3.2(3.5)
 19	2.5+/-0.6	3.2+/-1.0	 22	10.9(12.3)	10.0(12.3)
 20	2.8+/-0.8	3.8+/-1.4	 23	>1000	>1000
 24	2.1+/-0.3	2.8+/-0.5	 26	>1000	>1000
 25	4.1(9.4)	4.1(4.1)	 29	>333	>333
 27	4.2+/-2.5	1.7+/-0.9	 30	333	111
 28	3.3+/-2.6	1.7+/-0.8			

[a] DETERMINED BY RADIOLABLED OLIGONUCLEOTIDE-BASED ASSAY AS DESCRIBED IN THE EXPERIMENTAL SECTION. VALUES IN PARENTHESIS ARE THE RESULTS OF MULTIPLE RUNS.

Fig. 5A

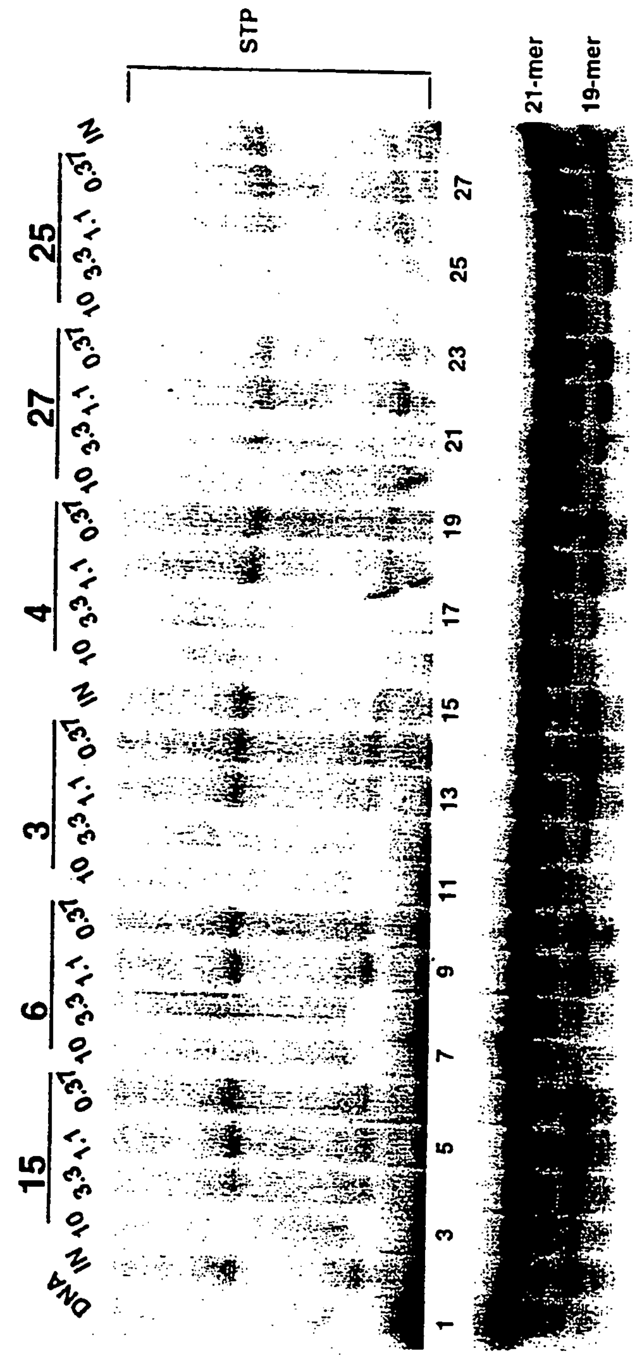

STP
21-mer
19-mer
DNA
15
6
3
4
27
25
IN
10 3.3 1.1 0.37
1
3
5
7
9
11
13
15
17
19
21
23
25
27

Fig. 5B

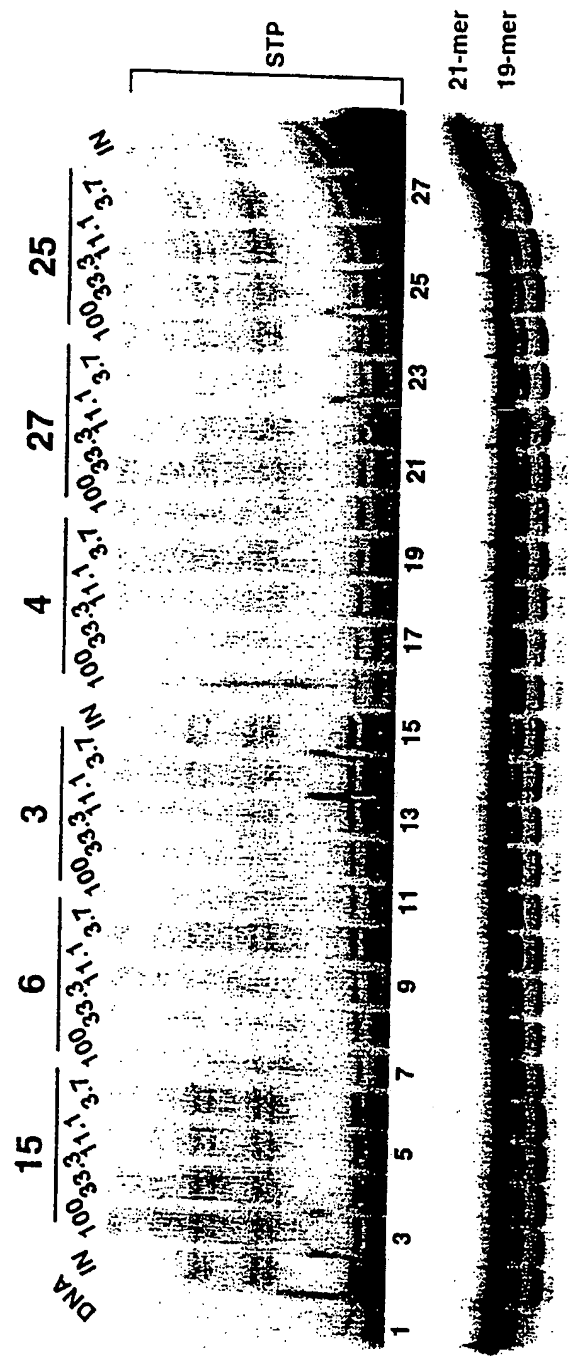

DNA
IN
15
6
3
4
27
25
100 33.3 11.1 3.7
STP
21-mer
19-mer
1 3 5 7 9 11 13 15 17 19 21 23 25 27

FIG. 5C

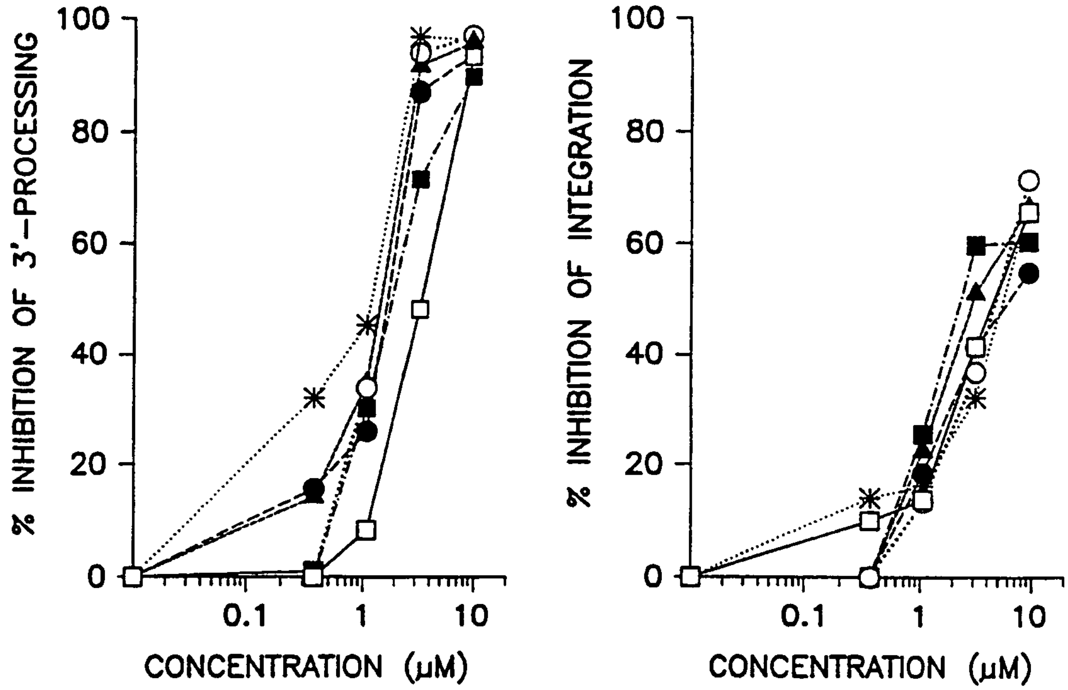
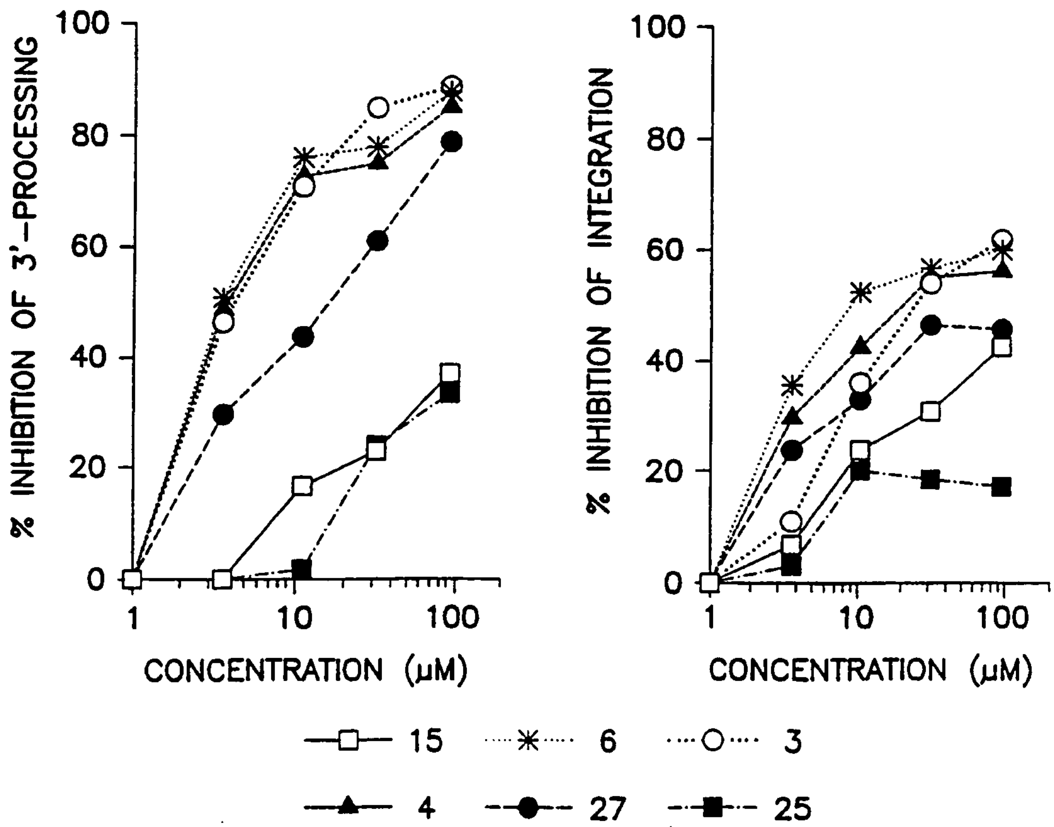

% INHIBITION OF 3'-PROCESSING
100
80
60
40
20
0
0.1
1
10
CONCENTRATION (µM)
% INHIBITION OF INTEGRATION
100
80
60
40
20
0
0.1
1
10
CONCENTRATION (µM)

FIG. 5D


% INHIBITION OF 3'-PROCESSING
100
80
60
40
20
0
1
10
100
CONCENTRATION (µM)
% INHIBITION OF INTEGRATION
100
80
60
40
20
0
1
10
100
CONCENTRATION (µM)
15
6
3
4
27
25

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/04608

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C69/732 C07C233/22 A61K31/216 A61K31/165

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data, BIOSIS, BEILSTEIN Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 99 48371 A (KING PETER J ;UNIV CALIFORNIA (US); REINECKE MANFRED G (US); ROBIN) 30 September 1999 (1999-09-30) the whole document ---	1,16-21
X	ROBINSON ET AL.: "Dicaffeoylquinic acid inhibitors ..." MOL PHARMACOL, vol. 50, no. 4, - 1996 pages 846-855, XP000916694 see compounds A,B,C,F,G,H ---	1,16-21
X	HE ZHAO: "Facile synthesis of ..." SYNTHETIC COMMUN, vol. 28, no. 4, - 1998 pages 737-740, XP000916763 see compounds 6 and 7 ---	1,16-21

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2000	16.08.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Goetz, G

3

Intl. ional Application No
PCT/US 00/04608

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KING ET AL.: "Structure-Activity Relationship ..." J MED CHEM, vol. 42, - 27 January 1999 (1999-01-27) pages 497-509, XP000919203 the whole document	1-21
P,Y	ROBINSON ET AL.: "Inhibitors of HIV-1 replication ..." PROC NATL ACAD SCI USA, vol. 93, - 1996 pages 6326-6331, XP000919204 the whole document	1-21
X	CHEMICAL ABSTRACTS, vol. 127, no. 13, 29 September 1997 (1997-09-29) Columbus, Ohio, US; abstract no. 173872, BUDZIANOWSKI, JAROMIR: "Coumarins, caffeoyltartaric acids, and their artifactual methyl esters from Taraxacum officinale leaves" XP000916756 abstract & PLANTA MED. (1997), 63(3), 288 , XP000916756	1
P,X	KAI ZHU ET AL.: "Irreversible Inhibition ..." JOURNAL OF VIROLOGY, 1 April 1999 (1999-04-01), pages 3309-3316, XP000916737 the whole document	1-21
P,X	LIN ET AL.: "Chicoric Acid Analogues as HIV-1 Integrase Inhibitors" J. MED. CHEM., vol. 42, 4 June 1999 (1999-06-04), pages 1401-1414, XP000919180 the whole document	1-21

3

International application No.
PCT/US 00/04608

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16,17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/04608

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9948371 A	30-09-1999	AU 3366899 A	18-10-1999